THE HYPOTHALAMIC-PITUITARY-TESTICULAR AXIS : STUDIES ON DELAYED PUBERTY AND INFERTILITY

FREDERICK CHUNG WEI WU

B.Sc.(Hons.), M.B. Ch.B., M.R.C.P.(U.K.)

M.D.

University of Edinburgh

1982

Dedicated to Joyce,
Robin, Anna and my parents

DECLARATION

In accordance with the statutes of the University of Edinburgh, I declare that the preparation and writing of this thesis has been carried out by myself. The research described in this thesis was conceived and conducted by myself except where the contribution of others is acknowledged.

Frederick Chung Wei Wu

ACKNOWLEDGMENTS

I wish to acknowledge the assistance of my colleagues in the studies submitted in this thesis. Hormone assays for testosterone were performed by Gail Hall; oestradiol and androstenedione by Ian Swanston, Department of Obstetrics and Gynaecology, University of Edinburgh. Luteinizing hormone and follicle stimulating hormone assays were undertaken in the laboratories of Dr W.M. Hunter, MRC Immunoassay Unit, Edinburgh, and the Regional Hormone Laboratory, Edinburgh by Brian Ogilvy, Janet McLaren and Keith Nicol. Prolactin was assayed in the laboratory of Dr A.S. McNeilly, MRC Unit of Reproductive Biology, Edinburgh. The sleep stage recording and analyses were performed by Sharron Borrow, Sleep Laboratory, Department of Psychiatry, Royal Edinburgh Hospital. I am most grateful to Professor I. Oswald for the facilities of the Sleep Laboratory. I am also grateful to Professor J.W. Farquhar, Department of Child Life and Health, University of Edinburgh for allowing me to study patients in his Paediatric Endocrine Clinic and for facilities of Ward 2, Royal Hospital for Sick Children, Edinburgh. I wish to thank Mr P. Edmond, formerly consultant urologist to the Royal Infirmary, Edinburgh for offering me the facilities of the Male Subfertility Clinic. The statistical analyses in Section B and Section C, chapter 2 were undertaken by Dr R. Elton and Mrs Gillian Raab respectively, of the Department of Medical Computing and Statistics, University of Edinburgh.

The help of Sister Ann Cook and Jenny Gray in obtaining some of the blood samples in these studies are gratefully acknowledged. I thank Dr G.A. Licoln and Professor R.V. Short, MRC Unit of Reproductive Biology, Edinburgh for their interest and helpful discussions. I also thank Alison Munro for her patient and efficient preparation of the manuscripts. Finally, I wish to express my deepest gratitude to my mentor, Professor D.T. Baird, for his constant encouragement and stimulation, for teaching me the art of clinical and scientific research, and for his unfailing guidance in general.

CONTENTS

ABSTRACT

The functional characteristics and control mechanisms of the hypothalamic-pituitary-testicular axis were explored by examining the patterns of pituitary and testicular hormone secretion in pubertal subjects and in adults with impaired spermatogenesis. The overnight hormone profile and the pituitary response to repeated GnRH stimulation were studied on 1-4 occasions in 16 patients (mean age 16.3 ± 2.7 years) with delayed sexual development while their clinical progress was followed for a mean duration of 22.4 ± 8.5 months. Changes in testis and genital size were confirmed to be the two physical markers which correlated best with the hormonal patterns. The earliest detectable evidence of pubertal onset was an increase in frequency and amplitude of pulsatile LH secretion during nocturnal sleep. Further progress in puberty was characterized by quantitative and qualitative changes in the pattern of LH secretion resulting from the combined effects of changing frequency of hypothalamic GnRH pulses, of gonadal feedback and possibly other ill-defined central mechanisms. Heightened pituitary responsiveness to GnRH could account for the pubertal rise in LH. Since the estimated amplitude of GnRH pulsatile secretion remained constant, the increase in GnRH pulse frequency was considered to be the prime mover in the activation of pituitary-testicular functions at the onset of puberty. Thus the hypothalamic GnRH frequency modulation of the pituitary provides the basic mechanism

for the neuroendocrine control of sexual maturation. The pituitary response to GnRH may be helpful in the endocrine assessment of patients with delayed puberty. In response to a single 10 μg bolus of GnRH, an LH rise of 5 u/l or more was associated with well-defined nocturnal pulsatile LH secretion in subjects who subsequently progressed through puberty spontaneously. However, in those where the GnRH-induced LH rise was under 5 u/l, it was not possible to differentiate between constitutional delayed puberty and hypogonadotrophic hypogonadism. The pubertal development of testicular functions was more closely related to the progressive increase in amplitude rather than frequency of pulsatile LH secretion. The roles of FSH and prolactin were less obvious. Exogenous GnRH-induced pulsatile LH secretion failed to stimulate testosterone production above the maximal levels in the morning. This implied that the pubertal Leydig cells normally function near to their maximal steroidogenic capacity.

In infertile patients, an inverse relationship between FSH and sperm count or the severity of germ cell depletion in testicular biopsies was confirmed. The application of FSH measurements to the assessment of 100 infertile patients was evaluated. Elevated FSH in those with sperm density under 5 million/ml indicated severe germ cell atrophy. The use of GnRH stimulation and multiple measurements of basal gonadotrophins and testosterone conferred no advantage. In patients with elevated FSH, circulating oestradiol, oestrone and

oestrone sulphate were significantly raised. There was preliminary evidence that the oestrogen excess resulted from testicular secretions of oestradiol. Oestrone sulphate was not secreted by the testis, being derived from unconjugated precursors - oestradiol and oestrone. The pathophysiological significance of these findings in male infertility and their relevance to normal intra-testicular control mechanisms were discussed.

In conclusion, the development and control of adult testicular functions are dependent on a background of pulsatile LH stimulation against which the complex interactions between FSH, Sertoli and Leydig cells ensure a continuous maturation of spermatogonia and the optimal production of testicular steroids.

SECTION A

I N T R O D U C T I O N

CHAPTER 1

HISTORICAL PERSPECTIVE

The study of human reproduction has long been hampered by religious, social, political and even scientific prejudice so that the most fundamental knowledge concerning the functions of gonads was not forthcoming until the late nineteenth century. The gonads, moreover, are unique amongst endocrine glands in that they subserve the dual functions of gametogenesis and sex hormone production. This has contributed to the complexity of the regulatory mechanisms of reproduction which are already burdened by the marked influence of reproductive cycles, age and extra-gonadal changes in the internal and external environments. Against these constraints, it is no surprise that the study of human reproduction had in the past failed to keep pace with advances in other branches of the life sciences. However, the rapid acceleration of world population growth after the 1950s and the technological advances in the field of endocrinology had brought about an intensification of scientific studies in reproduction and launched the discipline of reproductive endocrinology in the last two decades.

It is ironic that the importance of the testis in male virility has been recognised since ancient times. In the Neolithic age (7000 BC) when nomadic hunters turned to communal farming, animals first became domesticated by castration of male livestock (Steinach,

1940) - an ancient practice which remains evident in modern times. The use of human castration as a form of punishment for sexual offences has been recorded in texts dating back to the fifteenth century BC (Pritchard, 1955). The creation of eunuchs (Greek - eune : bed and echein : to hold or keep) by prepubertal castration for social purposes (harem attendants and slaves) was prevalent in China, India, Moslem Near East and ancient Greece (Spencer, 1946). In the Christian church, castration of boys to produce castrati male sopranos for church choirs was commonplace until this practice was terminated by Pope Leo XIII as recently as 1878 (Melicow & Pulrang, 1974). Aristotle (400 BC) studying the effects of castration in roosters and man, noted the differences between pre- and post-pubertal castration on secondary sexual characteristics (Thompson, 1910). Despite the long-recognised effects of castration, many centuries were to pass before the first experimental study on this phenomenon was made. Berthold (1849) showed that transplanting the testes from cockerels to long-term castrated capons in ectopic positions in the peritoneal cavity could restore the growth of the stunted comb and wattle as well as stimulating other masculine characteristics such as crowing and desire for mating. He concluded that the testes produced certain factors which can affect other tissues via the blood stream. This historical experiment was the first demonstration of the physiological action of any endocrine gland. Not only did this study mark the conception of male reproductive endocrinology but it also

ushered in the beginning of the science of endocrinology as a whole.

The importance of the testes in male fertility was in doubt for some 2000 years since Aristotle (400 BC) postulated that they merely acted as stabilizing weights to prevent the ducts from being drawn up within the abdomen. In 1668, de Graaf described the testes as being made up of minute convoluted tubules and correctly deduced that the fertilizing portion of semen was confected in the testes. Hamen, a student of Leeuwenhoeck, observed motile "animacules" in human semen under his teacher's simple microscope (Leeuwenhoeck, 1678). The significance of this observation was slow to be realized and the term spermatozoa embodied the prevailing opinion that these were parasites or symbiotic protozoa in the semen unrelated to fertility (Von Baer, 1827). Another student, Von Kölliker (1841), stimulated by the cell theory of Schleiden and Schwann, made a systematic search for the cellular origin of spermatozoa in 37 species of invertebrates. He concluded that spermatozoa arose from cellular divisions of the seminiferous tubules of the testis and were the most essential part of the semen. Following this, any remaining doubts regarding the significance of spermatozoa was dispelled by the demonstration that they enter and fuse with the female ovum (Barry, 1843; Hertwig, 1875). The gametogenic function of the testis was thus established.

After this inordinately long gestational period, the infantile discipline of male reproductive endocrinology

embarked on a slow prepubertal growth phase (ca 1850-1960). During this time, the classical extirpation and replacement experiments and fundamental biochemical studies of steroid and peptide hormones established the foundation for the more sophisticated studies on the endocrine regulation of the testis in the modern period. Although Leydig first described the clusters of epithelial cells in the interstices between the seminiferous tubules in 1850, it was over 50 years later that these cells were allocated the role of testicular hormone production on morphological and mainly circumstantial evidence (Bouin & Ancel, 1903). Using a lipoid extract of bull testes, Moore & McGee (1928) and Moore & Gallagher (1930) demonstrated reversal of the effects of castration on epididymal, seminal vesicular and prostatic functions in guinea pigs. Interestingly, it was not the appreciation of Berthold's or Moore's physiological studies but the popular misbelief that testicular failure led to symptoms of senility (Brown-Sequard, 1889) that stimulated the efforts to purify the active testicular principle. The first androgen to be identified was androsterone from urine (Butenandt, 1931). This was accomplished by the extraction of 15,000 litres of male urine yielding only 15 mg of crystalline androsterone. It soon became apparent that the testicular androgen was chemically distinct and much more potent than androsterone. Testicular testosterone was isolated in crystalline form by Laquer and colleages (David et al, 1935), its chemical formula identified and the hormone synthesized from

cholesterol in the same year (Ruzicka & Wettstein, 1935; Butenandt & Hanisch, 1935).

The first demonstration that the pituitary gland may be important in maintaining normal gonadal functions came from the simultaneous reports of Smith (1926) and Zondek & Aschheim (1926). These workers induced precocious ovarian development and sexual maturation in immature rats by daily implantation of fresh living rat pituitary fragments. Having also perfected the technique of selective surgical hypophysectomy by the ventral approach through the sphenoid sinus, Smith (1927 & 1930) was able to show that selective removal of the pituitary gland resulted in atrophy of the gonads, thyroid and adrenals. Daily implantation of living rat pituitary fragments led to complete cytological restoration in the latter three glands with mating and fertility in the hypophysectomized animals. This pioneering series of experiments not only proved that gonadal functions are normally controlled by the pituitary, but also established the concept of control of endocrine functions mediated by secretions from other endocrine glands. The search for an explanation as to how the pituitary can control both the follicular and luteal phases of ovarian function led to the fractionation of pituitary aqueous pyridine extracts into two distinct gonadotrophins, follicle stimulating hormone (FSH) and luteinizing hormone (LH) (Fevold et al, 1931). Extending these findings to the male, Greep et al (1936) showed that LH promoted the development and function of Leydig cells while FSH stimulated the

proliferation of the seminiferous epithelium in normal and hypophysectomized immature rats. The concept of dual pituitary control of male gonadal functions was thus formulated. However, other studies around that time indicated that androgens or LH could maintain spermatogenesis if replacement was commenced immediately after hypophysectomy (Walsh et al, 1934; Nelson & Merckel, 1938; Greep & Fevold, 1937). Hence, the exact physiological role of FSH has been cast in some doubt - an issue which remained unresolved for many years.

The effect of gonadal hormones on the pituitary was first described when Moore & Price (1932), somewhat to their surprise, produced atrophy of the seminiferous epithelium by administration of androgens to intact adult rats. This rather paradoxical finding was inspiringly interpreted as being due to the suppressive effect of excessive gonadal hormone on pituitary gonadotrophin secretion. Thus, one of the most fundamental dogmas in endocrinology, the negative feedback control of product on trophic hormone secretion, was introduced. Greep & Jones (1950) later confirmed that administration of testosterone proprionate lowered pituitary LH secretion in gonadectomized rats. The feedback control of FSH, in contrast, was much more difficult to establish. Martins & Rocha (1931) found that aqueous testicular extracts could prevent castration changes in the pituitary of castrated partners of intact/castrated parabiotic twin rats, while lipoid extracts in sufficient doses to maintain accessory gland functions had no effect. This

induced McCullagh (1932) and McCullagh & Walsh (1935) to confirm these findings and to postulate a second testicular hormone, inhibin, which was water-soluble. Acceptance of this hypothesis was hampered by the persistent failure to isolate and characterize this second active principle from the testis. Its total rejection, however, was prevented by a large body of evidence from experimental animals and infertile men with selective dysfunction of spermatogenesis which gave rise to a disproportionate increase in FSH secretion (for review, see Setchell et al, 1977).

The profound influence of external environmental stimuli, for example light, heat, food or season, on ovulation and the oestrous cycle has also aroused interest (Marshall, 1936). In species such as rabbit, ferret and mink, the so-called reflex ovulators, sensory stimulation of mating and orgasm are prerequisites for ovulation (Marshall, 1904; Heape, 1905). The central nervous system, being responsible for the perception and integration of environmental sensory input, could thus modify pituitary gonadotrophin secretion and reproductive function as a whole. This concept of neural regulation of reproduction was first subjected to experimental study when Marshall & Verney (1936) induced ovulation and pseudopregnancy in oestrous rabbits by passing a diffuse electrical current through the head. The close proximity of the adeno- and neurohypophysis in all vertebrates provided the necessary anatomical link between the anterior pituitary and the base of the brain. Further

studies then showed that electrical stimulation of the hypothalamus but not the anterior pituitary was followed by ovulation (Markee et al, 1946; Harris, 1948). This obligatory hypothalamic-pituitary relay was achieved within the hypophysial portal vessels (Green & Harris, 1947) rather than in the meagre neural supply between the neuro- and adenohypophysis. The neurovascular or neurohumoral mechanism of control of the anterior pituitary gained further impetus when extracts of hypothalamus were shown to be capable of inducing ovulation and releasing LH when infused directly into the pituitary or the systemic circulation (Campbell et al, 1961; McCann, 1962). Intensive study which followed led, a decade later, to the isolation and synthesis of porcine and ovine hypothalamic gonadotrophin releasing factor (GnRH) by the groups of Schally and Guillemin to whom the Nobel Prize for Medicine was jointly awarded. Sadly, the untimely death of Harris deprived him of the opportunity to witness the fruition of his pioneering work.

The stage leading up to the contemporary period is incomplete without another Nobel Prize winning achievement, this time by Yalow and Berson in the early 1960s. For a long time, progress in endocrinology has been held up by the non-availability of sensitive techniques for the measurement of low concentrations of hormones in body fluids. This obstacle was overcome by the technique of radioimmunoassay or radioligand assay (Yalow & Berson, 1960; Ekins, 1960). Utilizing the principle of competitive protein binding and the immunological

specificity of the antigen-antibody reaction, hormone measurements were carried out by comparing the effects of unknown samples with that of known standard solutions in their ability to competitively inhibit the binding of radio-labelled tracer hormones to their specific antibodies. These techniques enabled the accurate detection of pg amounts of hormones in minute quantities of body fluid and constitute the single most important methodologic advance in endocrine research and diagnosis.

In the ensuing sections, a more detailed account of the function and regulation of the hypothalamic-pituitary-testicular (HPT) axis, with emphasis on work in the last two decades, will be given.

CHAPTER 2

TESTICULAR STEROIDOGENESIS

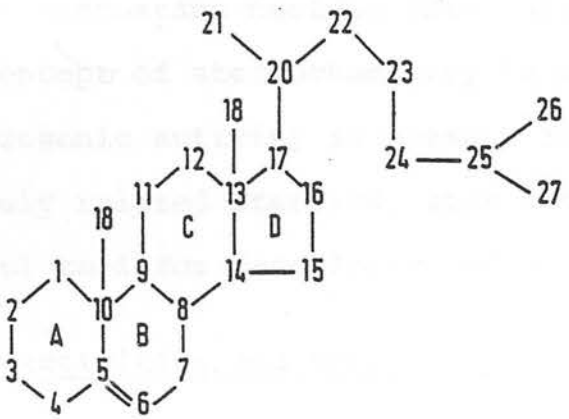
One of the two principal functions of the testis is the synthesis and secretion of androgens (Greek - aner : male, genos : descent).

2.1 Isolation

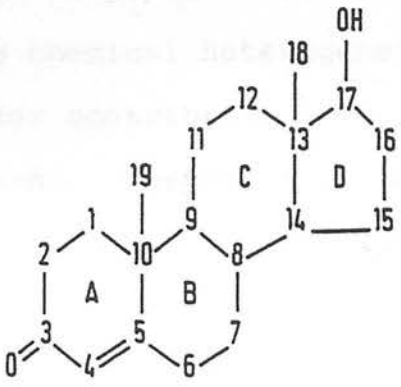
Due to the difficulty in preparation and the low yield of active extracts from testicular tissues, the first androgen, androsterone, was isolated from urine (Butenandt, 1931). However in 1935 David and co-workers successfully isolated crystalline testosterone from over 100 Kg of bull testes and in the same year the chemical structure was elucidated and synthesis of the hormone achieved (Rucizka & Wettstein, 1935; Butenandt & Hanisch, 1935). Thus from a relatively early stage pure testosterone has been available in sufficient quantity and purity for experimentation and clinical purposes.

2.2 Structure

Rosenheim & King in 1932 proposed the perhydro-cyclopentanophenanthrene formula of cholesterol, thus establishing the nuclear structure of the steroid molecule (Fig 2.1). The stereochemical relationship between gonadal steroid hormone and sterols was confirmed when Ruzicka et al (1934) showed that epicholestanol was degraded to androsterone. Estimation of the andro-genic potency of related steroid molecules demonstrated



CHOLESTEROL



TESTOSTERONE

Fig 2.1

The basic structure of the steroid molecule with the ring lettering and numbering of the carbon atom as in the cholesterol and testosterone molecules. The substituent groups at positions C_3 and C_{17} are critical determinants of the biological activities of testosterone.

that hormonal activity was dependent on the nature of the substituent groups and their stereochemical orientation around the basic polycyclic steroid nucleus. Hence, maximal androgenic activity was associated with 3-oxo and 17-β hydroxy substituents with a double-bond between C4-5 in the 19 carbon androstane nucleus (Fig 2.1). Although such a simple concept of stereochemistry is no longer tenable and androgenic activity is present in a large variety of loosely related steroids, this has nevertheless remained a useful tool for descriptive purposes at least.

2.3 Biological activities and relative potency

Like other low molecular-weight terminal chemical messengers (for example, thyroid hormones and catecholamines) testicular androgens have an extensively distributed population of target sites and control diverse metabolic and physiological processes (q.v.). This contrasts sharply with the relatively restricted target trophism of peptide hormones such as gonadotrophins. The chemical heterogeneity of androgens probably further contributes to this diversity of action and target organs. Despite this, androgens are often defined by their main biological properties of evoking the attributes of maleness rather than by chemical structures. This classical definition is necessarily limited by the available bioassays - for example, stimulation of comb growth in fowls and seminal vesicle and prostate weight gain in rodents, which does not take into account the broader concepts of androgen functions

including the promotion of in-utero development of the male genital tract (Jost, 1953), trophic effect on spermatogenesis (Steinberger, 1971) and the control of male sexual behaviour (Perloff, 1949). Androgens are also known to have extra-reproductive actions such as trophic effects on the kidney, liver and thymus, nitrogen retention and protein anabolism, red blood cell regeneration and influence on non-sexual hair metabolism leading to frontal balding (for review, see Dorfman & Shipley, 1956). Most of these androgenic functions are difficult to define and quantitate so that for the purpose of assessing relative potency of putative androgenic compounds, the use of capon and rodent assays are still standard procedures even though this is not strictly correct. As assessed by these two biological end-points, testosterone and dihydrotestosterone are by far the most potent androgens. Recent progress in the understanding of the mechanism of androgen action and the conversion of androgens to more active metabolites by the target cells further emphasize the limitation of the classical concepts of relative potencies.

2.4 Biosynthesis of testicular steroids

Qualitatively, steroidogenic pathways in the testis, adrenal, ovary and placenta are essentially similar. Most of the earlier studies however were conducted by the use of radio-labelled substrates in in-vitro perfusion or incubation experiments. These were unable to yield quantitative information regarding the contribution and

relative importance of specific intermediates or pathways. In the last decade, the ability to isolate specific cellular and subcellular components of the testes (q.v.) and the improved sensitivities of radioimmunoassays and mass spectrometry have enabled the measurement of endogenous steroid levels and production rates in isolated testis cellular preparations and subcellular components yielding much greater insights into the mechanisms and biological significance of various steroidogenic pathways.

2.4.1 Cholesterol and Pregnenolone

The testis synthesizes testosterone from acetate which is converted to cholesterol by a series of reactions involving carbon chain elongation, folding and ring closure and intermediates of mevalonic acid, farnesyl pyrophosphate, squalene and lanosterol (Tsai et al, 1964; Nightingale et al, 1967). The mechanism of conversion of cholesterol to pregnenolone was primarily established in the adrenals but the reaction sequence is generally acceptable for the gonads as well. The hydroxylation of cholesterol involves a simultaneous oxygenation of cholesterol at C_{20} and C_{22} forming delta 20-22 cholesterol, 20,22-epoxycholesterol and 20α,22R-dihydroxycholesterol (Kraaipoel et al, 1975a and b). The last compound is then converted by the side-chain cleavage enzyme C_{20-22} lyase to pregnenolone and the 6-carbon isocaproic acid. This series of reactions requires NADPH, oxygen and an electron transport system containing a flavoprotein, noneheme iron protein and

cytochrome P450 (Hall & Shikita, 1974). Pregnenolone is the starting point for the specific enzymatic pathways for the synthesis of all steroid hormones.

2.4.2 Testosterone

Pregnenolone is converted to testosterone via either a Δ_4 (Slaunwhite & Samuels, 1956) or Δ_5 pathway (Oertel & Eik-Nes, 1962). The Δ_4 pathway involves the conversion of pregnenolone to progesterone by 3β-hydroxysteroid dehydrogenase and 3β-5-ene-ketosteroid isomerase, hydroxylation of progesterone to 17α-hydroxyprogesterone by 17α-hydroxylase, cleavage of a 2-carbon side chain from 17α-hydroxyprogesterone to androstenedione by C_{17-20} lyase and the reduction of androstenedione to testosterone by 17β-hydroxysteroid dehydrogenase (17 ketosteroid reductase).

Conversion of pregnenolone to testosterone by the Δ_5 pathway involves the hydroxylation of pregnenolone to 17α-hydroxypregnenolone by 17α-hydroxylase, cleavage of side chain of 17α-hydroxypregnenolone by C_{17-20} lyase to form dehydroepiandrosterone, which is then converted to androstenedione by 3β-hydroxysteroid dehydrogenase and isomerase and the androstenedione converted to testosterone by 17β-hydroxysteroid dehydrogenase. An alternative route in the Δ_5 pathway is the conversion of dehydroepiandrosterone to androstenedione by 17β-hydroxysteroid dehydrogenase before conversion to testosterone by 3β-hydroxysteroid dehydrogenase and isomerase (Fig2.2).

In humans, the Δ_5 pathway is predominant for the synthesis of testosterone (Axelrod, 1965; Bell & Lacy,

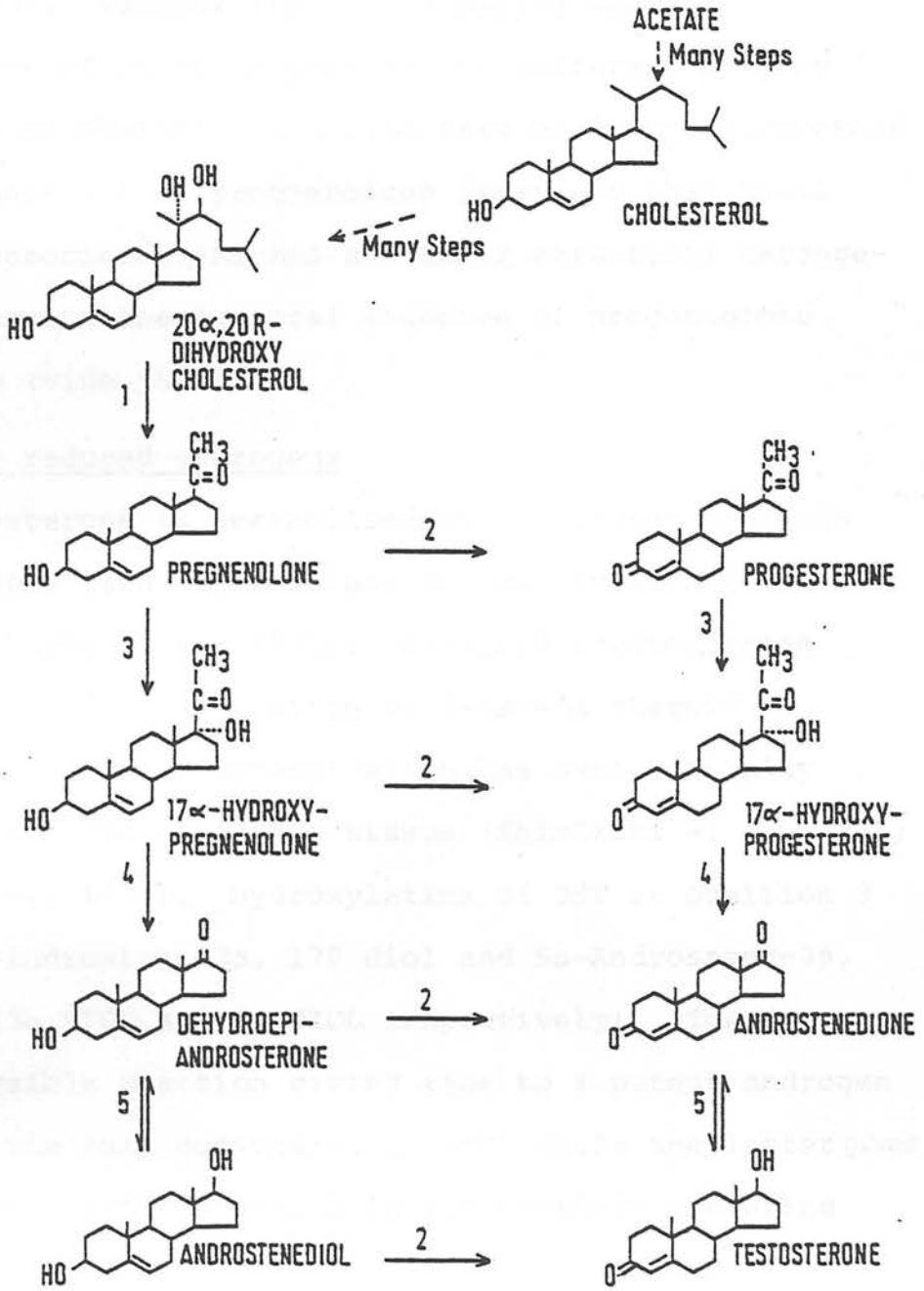


Fig 2.2

The pathways of testosterone biosynthesis from acetate and cholesterol. The many steps in the synthesis of cholesterol from acetate and the oxidation of cholesterol are not shown. The formation of pregnenolone involving the side-chain cleavage enzyme complex C_{20-22} lyase (1) is the starting point of steroid hormone synthesis. The righthand column shows the "Δ^4 pathway", the lefthand column the "Δ^5 pathway". The enzymes involved in the various steps are isomerase and 3β-hydroxysteroid dehydrogenase (2), 17α-hydroxylase (3), C_{17-20} lyase (4) and 17β-hydroxysteroid dehydrogenase.

1974; Yanihara & Troen, 1972) although there is evidence that the Δ_4 pathway is not entirely redundant (Steinberger et al, 1973). Tamaoki (1973) speculated that the predominance of Δ_5 or Δ_4 pathways in different species may depend on whether 17α-hydroxylase or 3β-hydroxysteroid dehydrogenase act on pregnenolone first and that these membrane-associated enzymes and their structural arrangements determine the temporal sequence of pregnenolone metabolism (vide infra).

2.4.3 5α- reduced androgens

Testosterone is metabolized to 5α-reduced products in the testis (and other sites) in many species (Rose et al, 1973; Payne et al, 1973). 5α-dihydrotestosterone (DHT) is formed by the action of 3-oxo-5α steroid Δ_4-dehydrogenase (5α-reductase) which has been partially purified from rat prostatic tissue (Shimizaki et al, 1973; Taurog et al, 1975). Hydroxylation of DHT at position 3 yields 5α-Androstane-3α, 17β diol and 5α-Androstane-3β, 17β diol (3α DIOL and 3β DIOL respectively). The former is a reversible reaction giving rise to a potent androgen (possibly via back conversion to DHT) while the latter gives a much weaker androgen which is irreversibly converted (Robel et al, 1971) (Fig 2.3).

2.4.4 Oestrogens

Although androgens are qualitatively and quantitatively the most important steroids synthesized in the testis, the presence of oestrogens in the human testis

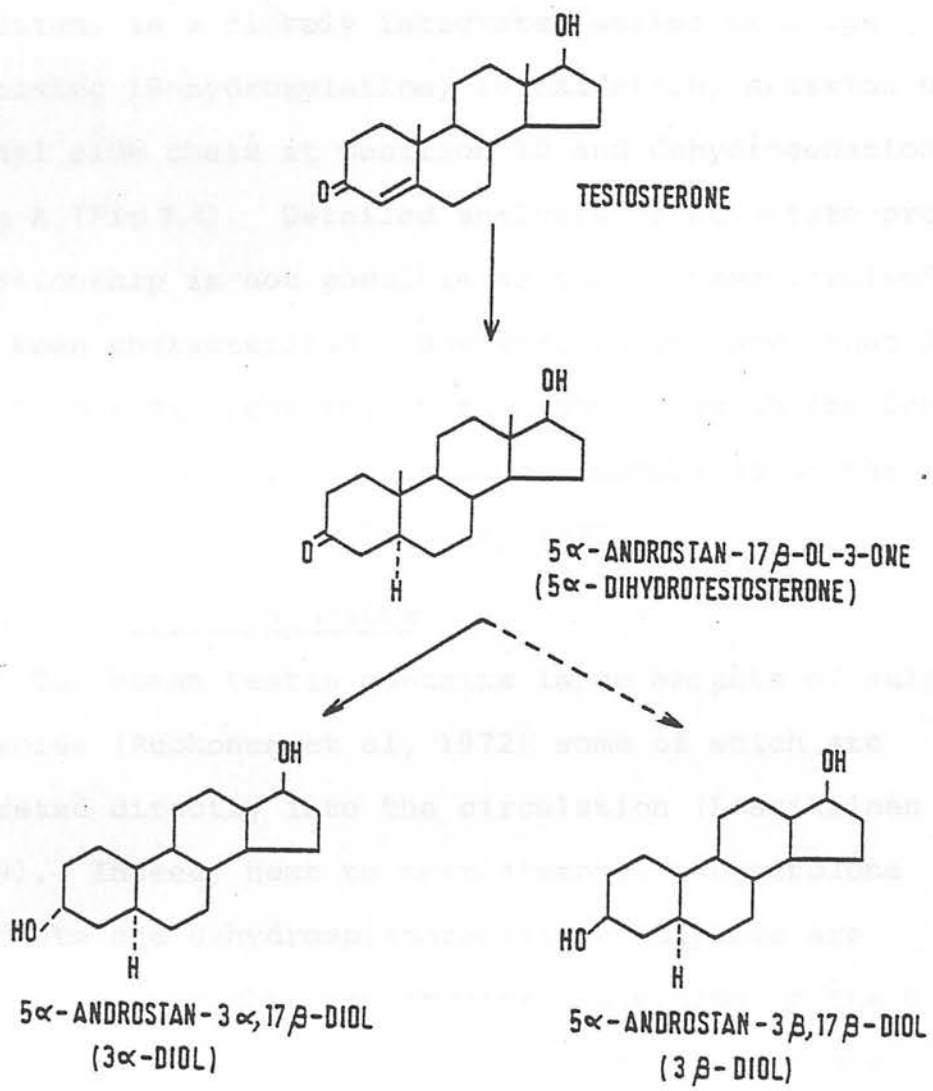


Fig 2.3

The formation of 5α-dihydrotestosterone and 3α and 3β- Diols from testosterone by the enzymes 5α-reductase and 3-ketosteroid reductase respectively.

has been known for a long time (Goldzieher & Roberts, 1952). Indeed, the stallion testis is the richest known biological source of oestrogen (Beall, 1940). As in the ovary and placenta, the substrates for oestrogen synthesis are testosterone and androstenedione. The conversion of androgen to oestrogen, known as the aromatization reaction, is a closely integrated series of steps involving 19-hydroxylation, 18 oxidation, scission of the methyl side chain at position 10 and dehydrogenation of ring A (Fig 2.4). Detailed analysis of substrate-product relationship is not possible as the enzymes involved had not been characterized. However, it is known that NADPH and oxygen are required by the enzymes which are located in the smooth-surfaced microsomal membranes of the stallion testis (Oh & Tamaoki, 1973).

2.4.5 Steroid conjugates

The human testis contains large amounts of sulphated-steroids (Ruokonen et al, 1972) some of which are secreted directly into the circulation (Laatikainen et al, 1969). Indeed, next to testosterone, pregnenolone sulphate and dehydroepiandrosterone sulphate are quantitatively the most important steroids in the human testis (Payne et al, 1973). A number of sulphated steroids including pregnenolone can act as substrates for testosterone synthesis by the human testis (Payne et al, 1971; Payne & Jaffe, 1975; Yanihara & Troen, 1972). It has been postulated that the human testis may utilize a pathway of testosterone synthesis involving sulphated substrates and intermediates (Ruokonen, 1978).

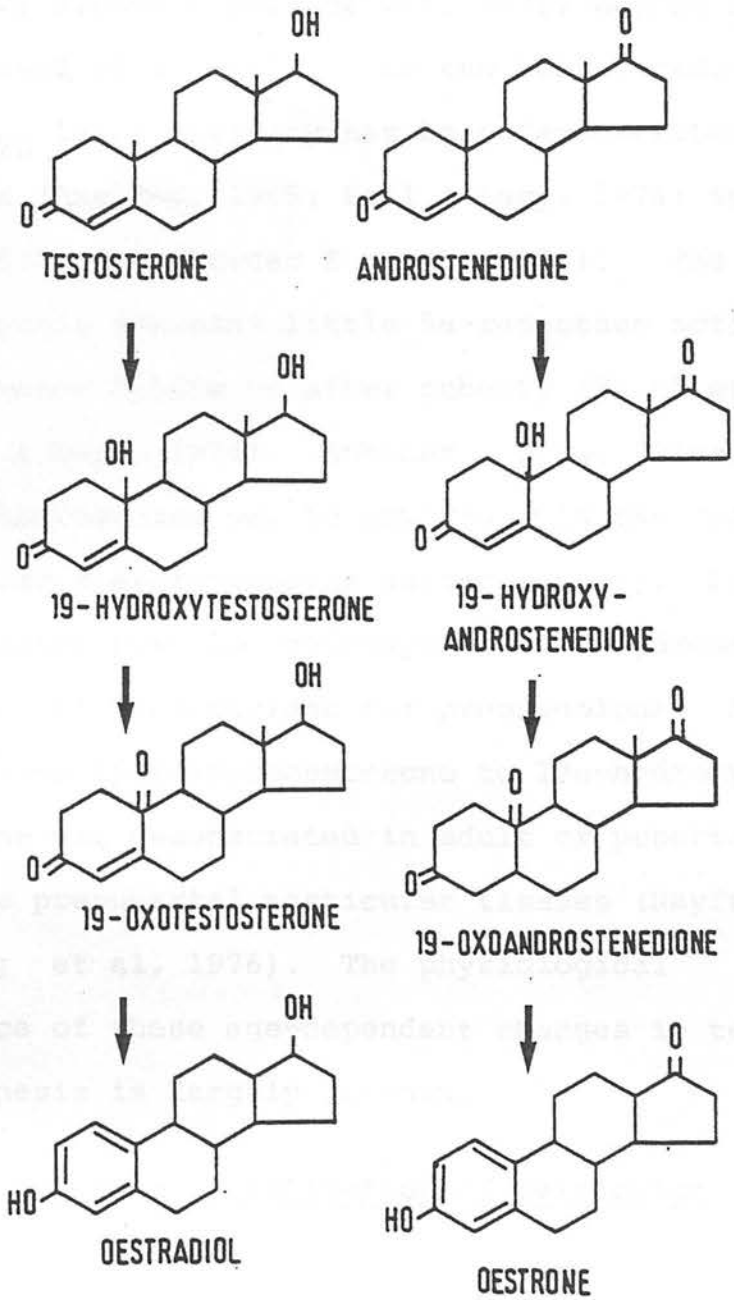


Fig 2.4

The formation of oestrogens from androgens in the aromatization sequence of reaction.

2.4.6 Age and testicular steroidogenesis

Studies in the rat have demonstrated a marked age-dependency of testicular steroidogenic activity (Rivarola et al, 1972; Ficher & Steinberger, 1971; Nayfeh et al, 1966; Mizutani et al, 1977). In the human, reduced in-vitro C_{17-20} lyase activity has been demonstrated in the aged testis (Axelrod, 1965; Bell & Lacy, 1974; Steinberger A. et al, 1970; Steinberger E et al, 1970). The pre-pubertal testis contains little 5α-reductase activity which increases during or after puberty (Kelch et al, 1971; Bell & Lacy, 1974). Another enzyme, 20α-hydroxy-steroid dehydrogenase may be important in the regulation of testicular steroidogenesis during puberty. Fan et al (1974) proposed that 20α-hydroxysteroid dehydrogenase may compete with 17α-hydroxylase for pregnenolone. A higher ratio of 20α-dihydroxyprogesterone to 17α-hydroxy-progesterone was demonstrated in adult or pubertal as compared to prepubertal testicular tissues (Nayfeh et al, 1975; Berg et al, 1976). The physiological significance of these age-dependent changes in testicular steroidogenesis is largely unknown.

2.5 Intratesticular localization of testicular steroidogenesis

Having established some of the qualitative and quantitative aspects of testicular steroidogenesis, the understanding of testicular function can be extended by the localization of these enzymatic processes to particular cell types or subcellular organelles. Most

steroidogenic enzymes are particulate or membrane-bound and purification and solubilization have not been achieved. To gain further insight into the functional characteristic and molecular mechanisms of specific steps in the steroidogenic pathway, the study of structure-function relationships is essential.

2.5.1 Cellular localization

The recognition that androgens are important in the regulation of spermatogenesis (Steinberger, 1971) makes it imperative to clarify the exact intratesticular cellular source of androgen production. The definitive experiments have only been accomplished relatively recently considering that Leydig cells were implicated to be the site of androgen production since the 1900s. Having overcome the practical obstacle of separating seminiferous tubules from the interstitial tissue in rat testes, Christensen & Mason (1965) found that conversion of ^{14}C-progesterone to androstenedione and testosterone was two to three times higher in the interstitial tissue. The seminiferous tubules had a low but definite capacity for androgen conversion from progesterone. Considering that cholesterol is a more physiological substrate than progesterone, Hall et al (1969) repeated the same experiment using ^{14}C-cholesterol. It was found that interstitial tissues converted cholesterol to testosterone while seminiferous tubules were unable to do so. The obvious conclusion however was coloured by the fact that cholesterol is rather insoluble and the intact blood-testis-barrier may prevent the entry of substrate into

the seminiferous tubules. To answer these criticisms, Cooke et al (1972), using a sensitive radioimmunoassay to determine endogenous testosterone production in vitro, found that seminiferous tubules were unable to synthesize testosterone de novo while testosterone concentration increased in interstitial tissue and whole testis incubates. It is now generally accepted that testicular androgen synthesis occurs predominantly or solely in Leydig cells under physiological conditions, but the potential contribution of seminiferous tubular androgen derived from progesterone is unknown.

In contrast to testosterone, the 5α-reduced androgens are preferentially produced in the seminiferous tubules (Payne et al, 1973; Rivarola et al, 1972). Sertoli cell-enriched preparations were the most efficient in conversion of ^{14}C-DHT to 3α-DIOL while the spermatocytes were best at producing DHT from testosterone (Dorrington & Fritz, 1975).

The cellular origin of testicular oestrogen is contentious. Early evidence (reviewed in Armstrong & Dorrington, 1977) suggested that both Sertoli cell and Leydig cell tumours were capable of oestrogen synthesis. Direct experimental demonstration of Sertoli cell synthesis of oestrogen was afforded by de Jong et al (1974). They found that oestradiol concentration, though lower in the seminiferous tubules initially, increased during incubation while that in interstitial tissues remained unchanged. Sertoli cell-enriched cultures from immature rats were capable of converting added testo-

sterone and androstenedione to oestradiol in the presence of FSH (Dorrington & Armstrong, 1975). However, two groups reported that the site of aromatase activity in human (Payne et al, 1976) and rat testis (Canick et al, 1979; Valladares & Payne, 1979) is located in Leydig cells which are responsive to hCG but not FSH. The relative importance of these two possible intratesticular sources of oestrogen remains to be clarified but it is likely that the cellular origin of testicular oestradiol is critically dependent on the age of the animal (Pomerantz, 1979).

2.5.2 Subcellular localization

Steroidogenesis de novo can be demonstrated in cell-free homogenates of steroid secreting tissues (Tamaoki, 1973) suggesting that the cell structure itself is not crucial to steroidogenesis but the subcellular organization of steroid secreting cells is of importance. It is thus necessary to relate steroidogenic function to subcellular organelles. Testicular steroidogenic enzymes are tightly bound to membrane structures and particles after subcellular fractionation with minimal activity escaping into the supernatant or cytosol fraction. Enzymes for the side-chain cleavage of cholesterol are located in the inner membranes of mitochondria (Moyle et al, 1973; Van der Vasse et al, 1974) while the rough-surfaced microsomal fraction of endoplasmic reticulum contained the various enzymes for conversion of pregnenolone to testosterone and oestradiol (Tamaoki, 1973; Van er Molen et al, 1975). This implies that a

system for the intracellular transport of steroidogenic intermediates analogous to the intercellular transport of steroids must exist. Hence, cholesterol esters are hydrolysed to free cholesterol in the cytoplasmic lipid droplets and then transported to the mitochondria for side-chain cleavage. The pregnenolone thus produced has to pass out of the mitochondria to reach the surface of the agranular endoplasmic reticulum to be converted to testosterone. The mechanism of this intracytoplasmic substrate transfer is unknown but recent evidence suggests that the action of LH on steroidogenesis may be upon this transport system (q.v.). The compartmentalization of enzyme complexes and the close association of enzyme proteins on or in biomembranes of subcellular organelles also raise the possibility that the physicochemical organization of enzyme-membrane structures may provide a local mechanism regulating the temporal sequence of enzyme reactions. In other words, microsomal enzymes do not behave like enzymes in a homogeneous system with a uniform distribution of activity. Local physical interaction between substrate and enzyme can override the substrate preference of different enzymes in the same membrane. Evidence for this mechanism was convincingly argued by Tamaoki (1973) and Samuels et al (1975). They postulated that microsomal enzymes are organized on or in the membranes in a genetically-determined pattern so that the series of enzyme reactions are performed in a fixed sequence which cannot be altered by factors such as cofactors, substrate concentration or

substrate affinity.

The cellular and subcellular compartmentalization of steroidogenic enzymes provide multiple opportunities for the spatial regulatory mechanisms to operate. These mechanisms may serve to restrict the huge number of steroids that can theoretically be synthesized and also to enhance the production of the few steroids that have evolved phylogenetically to be the most important for the reproductive functions of a particular species.

CHAPTER 3

CONTROL OF TESTICULAR STEROIDOGENESIS

The synthesis and secretion of androgens by the testis are stimulated by LH in intact animals (Eik-Nes, 1971; Odell et al, 1974), in isolated testicular tissue (Cooke et al, 1972; de Jong et al, 1974), isolated interstitial tissue (Cooke et al, 1972; de Jong et al, 1974) and in purified Leydig cells (Janszen et al, 1976; Purvis et al, 1978). The mechanisms of control of Leydig cell function is a topic that in the last few years has yielded a profusion of new data which has somewhat outpaced their assimilation into the existing concepts of testicular function as a whole. A number of different mechanisms can theoretically be involved in the control of steroid synthesis - for example, substrate or cofactor availability, compartmentalization of substrate and enzymes, the induction, inhibition and stimulation of enzyme activities. Most investigators have concentrated on the activation or inhibition of enzyme systems and it is to these parameters that most of the recently postulated molecular mechanisms have been related. Although LH is undisputedly the prime trophic hormone for Leydig cells with an obligatory role, it should be emphasized that other factors, local as well as systemic, also contribute to the overall physiological control of Leydig cell steroidogenesis at different stages of testicular function.

3.1 Positive regulation of Leydig cells

3.1.1 Morphological effects of LH/hCG

There is agreement that chronic LH/hCG treatment results in Leydig cell hypertrophy, increase in sub-cellular organelles associated with steroidogenic and secretory processes (mitochondria, endoplasmic reticulum and Golgi apparatus), cytoplasmic lipid depletion and changes in nuclear shape and size (de Kretser, 1967).

The question of hyperplasia in response to LH/hCG stimulation is more controversial. Although hCG treatment in immature (Chemes et al, 1976) and adult rats (Christensen & Peacock, 1980) gave rise to increases in Leydig cell numbers, this does not seem to be the case in normal adult men (Heller & Leach, 1971).

3.1.2 Effect of LH/hCG on the steroidogenic pathway

Commensurate with the observed increase in sub-cellular organelles LH/hCG increases or maintains the activity of 3β hydroxysteroid dehydrogenase, 17α-hydroxylase and 17-20 lyase (see Hall, 1970; Ewing & Brown for review), probably as part of a generalized trophic effect on Leydig cells. However it is generally accepted that LH has a specific stimulatory action on the rate-limiting step of cholesterol side-chain cleavage forming pregnenolone (Menon et al, 1967; Hall, 1970). This may be effected by the activation of the mitochondrial enzyme complex including cytochrome P450 system and NADPH. Other possible LH-mediated actions may involve the hydrolysis of cholesterol esters to free cholesterol (Moyle et al,

1973) and the transport of cholesterol to mitochondria by cytoskeleton microfilaments (Hall et al, 1979).

3.1.3 Mechanism of positive regulation of steroidogenesis by LH/hCG

It is well established that specific hormone binding to cell membrane receptors is the first step in the mechanism of LH action on its principal target cell in the testis, the Leydig cells. The presence of these receptors and their specific binding to ^{125}I-labelled LH after in vivo and in vitro exposure was first demonstrated in the rat (de Kretser et al, 1969 & 1971). Similar binding of hCG in the human adult (Hsu et al, 1978) and foetal testis (Huhtaniemi et al, 1977; Frowein & Engel, 1974) has since been confirmed. Further characterization of the binding sites revealed specific receptors in the Leydig cell membrane (Mendelson et al, 1975) with high affinity (Ka 2.4 x 10^{10} M^{-1}) and low capacity (10^{-12} mol/G or 20,000 sites/Leydig cell) for LH (Catt et al, 1972a & b). LH binding to Leydig cell membrane receptors activate the inner membrane associated adenyl cyclase activity resulting in net formation of cyclic AMP (Rommerts et al, 1974; Catt & Dufau, 1976). Dose response study of receptor-hormone interaction showed that occupation of under 1% of total receptor sites was sufficient to stimulate maximal steroid production. Thus maximum hCG binding by rat testis was reached at a hormone concentration of 200 ng/ml whereas maximum steroidogenesis could be stimulated by only 0.5 ng/ml of hCG (Catt & Dufau, 1973; Catt et al, 1974). The concept

of spare receptors, which originated in pharmacological studies of drug responsive tissues (Stephenson, 1956), was therefore introduced into the study of hormone-receptor interaction. The physiological significance of spare receptors, despite many speculations, is still uncertain.

Cyclic AMP increases testosterone synthesis in the testis (Sandler & Hall, 1966a & b; Connell & Eik-Nes, 1968) and is regarded as an obligatory intracellular second messenger of LH stimulation. Although some discrepancy was shown between the amount of LH required to stimulate testosterone synthesis and the intracellular rise in cyclic AMP, especially at low gonadotrophin concentrations, Dufau et al (1977) were able to confirm that testosterone synthesis in response to hCG stimulation in rat Leydig cells was paralleled by a significant increase in bound rather than total cyclic AMP. The sole mechanism by which cyclic AMP acts within eukaryotic cells is via activation of cyclic AMP-dependent protein kinases. These are widespread enzyme systems that transfer the phosphate from ATP to a substrate - serine or threonine hydroxyl groups in proteins which become "activated" by phosphorylation (Rubin & Rosen, 1975 for review). Cyclic AMP binds to the regulator subunit of the inactive holoenzyme and releases the active catalytic subunit of protein kinase. Evidence that these mechanisms are operative in LH stimulation of Leydig cell was provided by Cooke et al (1975, 1976). It is however not clear how the activated protein kinase will influence

cholesterol side-chain cleavage, which is the accepted rate limiting step in steroidogenesis. The phosphorylation of endogenous proteins (Cooke et al, 1977), stimulation of overall protein synthesis (Cooke et al, 1975) and specific protein synthesis (Janszen et al, 1978) may in some way activate the mitochondrial enzymes. The functional properties of these LH-dependent proteins are unknown.

The above series of membrane receptor-triggered reactions is typical of peptide or non-steroid hormone. Their possible physiological significance has only been related to steroidogenesis. Whether the same mechanisms mediate the chronic trophic effects seen after LH/hCG stimulation (q.v.) is entirely unknown.

3.1.4 Functional heterogeneity of Leydig cells

Studies on biochemical mechanisms in isolated cell populations can only be meaningful if functionally homogeneous cell populations are used. Unfortunately, it has been repeatedly confirmed that within a morphologically homogeneous population of interstitial cells, functional differences can be recognized (Rommerts & Brinkman, 1981). Different steroidogenic responses to LH in the various populations of Leydig cells separated under a variety of conditions (Payne et al, 1980a & b; Rommerts et al, 1980) have recently been reported. The physiological basis of the functional differences in Leydig cells, the transformation between different cell types and their possible interactions are but some of the fundamental questions still to be answered.

3.2 Negative regulation of Leydig cells

The loss of target cell receptors inversely related to circulating hormone concentration was first described for insulin and growth hormone (Roth et al, 1975) and has since been reported in numerous tissue systems, e.g. catecholamines, TRH and epidermal growth factor. The loss of LH receptors initiated by the occupation of a small proportion of the receptor population following the single injections of the homologous hormone (Hsueh et al, 1976; Sharpe, 1976) conforms to this general pattern of ligand regulation of cell surface receptor concentration. This dose dependent phenomenon is maximal at 2-4 days, returning to normal after 6-8 days. Receptor occupancy became undetectable 24-36 hours after hormone injection and therefore cannot account for the later loss of much larger numbers of LH receptors (Tsuruhara et al, 1977). The loss of LH receptors is thought to occur by internalization of hormone-receptor complexes and also unoccupied receptors (Catt et al, 1979).

The gonadotrophin-induced loss of LH receptors in Leydig cells is accompanied by impaired cyclic AMP and reduced steroidogenic response to further stimulation by hCG (Hsueh et al, 1977; Sharpe, 1977). This desensitization or refractoriness however can be dissociated from loss of LH receptors (Haour & Saez, 1978) and at low hormone concentrations, an enhancement of maximal testosterone production to hCG could be associated with loss of LH receptors (Dufau et al, 1978). However, large single doses of hCG reduce the capacity of Leydig cells

to synthesize testosterone (Hsueh et al, 1977; Sharpe, 1977) independently of receptor changes (Tsuruhara et al, 1977). This gonadotrophin induced steroidogenic lesion has been localized to post-cyclic AMP steps most probably involving blocks in the action of 17α-hydroxylase and 17-20 lyase (Tsuruhara et al, 1977; Cigorraga et al, 1978; Chasalow et al, 1979), which become further possible rate limiting steps for testosterone production. There is some evidence that similar lesions may occur in men (Padron et al, 1980; Wang et al, 1980). The similarity of this gonadotrophin induced steroidogenic block to that of the direct suppressive effect of oestradiol on Leydig cells (Samuels et al, 1969; Kalla et al, 1980; Brinkman et al, 1980) has led to the suggestion that testicular oestradiol may mediate this desensitization process. The observation that tamoxifen, an oestrogen antagonist, can abolish the gonadotrophin-induced desensitization of rat Leydig cells further substantiates this theory (Cigorraga et al, 1980).

It has been shown that GnRH or its hyperactive analogues were able to bring about almost identical changes in LH receptors and steroidogenic enzyme activities as observed after hCG injection in intact animals (Dufau et al, 1979; Berlanger et al, 1980). Similar changes have also been described in man (Bergquist et al, 1979). When these same changes could be reproduced by GnRH in hypophysectomized animals (Hsueh & Erickson, 1979; Bambino et al, 1980) the question of a direct testicular action of GnRH was considered. This possibility

was strengthened by the demonstration of specific, high affinity receptors for GnRH and its analogues on rat Leydig cells (Clayton et al, 1980; Sharpe & Fraser, 1980a) and the secretion by rat Sertoli cells of a "GnRH-like" factor which, though immunologically distinct from GnRH, can stimulate LH release from pituitary tissue (Sharpe et al, 1981; Sharpe, 1982). Increased levels of this "GnRH-like" factor was stimulated by hCG (Sharpe & Fraser, 1980b) leading to speculations that this peptide molecule could mediate some of the hCG-induced changes in the Leydig cells.

Despite the considerable amount of new information briefly outlined above, our understanding of the physiological control of Leydig cell function by LH remains rather poor. Virtually all the mentioned studies used single injections of pharmacological doses of LH/hCG in in-vitro studies of isolated Leydig cells and it is debatable whether the apparent negative hormone influence by receptor down regulation or steroidogenic block has any physiological relevance. However, several studies with small doses (100-200 ng/animal) of hCG (Tsuruhara et al, 1977; Sharpe, 1977; Dufau et al, 1978) did demonstrate loss of LH receptors independent of the effect on steroidogenesis in Leydig cells. These concentrations were compatible with normal physiological extremes and that observed with pulsatile secretion of LH. On the other hand, only 1% of the total receptor population is required for maximal activation of steroidogenesis. This minute degree of receptor occupancy and turnover and an

overwhelming abundance of spare receptors may be a mechanism which ensures maximum sensitivity of the Leydig cells by maintaining a fairly stable surface receptor concentration available for LH binding under the extremes of the physiological range (Catt et al, 1980). If this is true, then receptor down regulation by the homologous hormone may serve to limit the sensitivity of response when Leydig cells are exposed to high hormone concentrations. Nevertheless, it seems likely that under physiological conditions, the negative effects of LH are subtle and only become detectable when they are magnified by experimental conditions or in pathological situations. Thus in patients with chorioncarcinoma of the testes, circulating LH reaches extremely high levels but plasma testosterone remains within the normal range (Kirschner, 1970). In testicular feminized (TFM) mice the testes are exposed to high LH concentrations. In spite of the Leydig cell hyperplasia in these testes, LH receptor content remains reduced and their sensitivity to LH stimulation in vitro is similarly impaired (Purvis et al, 1978). It is obvious that further work, with special reference to reproducing physiological conditions, will improve our understanding of Leydig cell function and control.

3.3 Follicle stimulating hormone

Although the classical theory of dual gonadotrophin control of the testes ascribed no steroidogenic function to FSH, some recent evidence suggested that it may have a

synergistic action with LH on Leydig cell function (Connell & Eik-Nes, 1968; Lostroh, 1969). Direct evidence was first provided by the finding that FSH potentiated the effect of LH on testicular secretion in the perfused adult rabbit testis (Johnson & Ewing, 1971). In immature rats, the early rise in FSH was associated with an increase in the number of LH receptors on the Leydig cells (Odell & Swerdloff, 1976; Ketelslegers et al, 1978). In adult hypophysectomized rats with regressed testes, FSH alone or in synergism with LH can increase testosterone secretion (El Safoury & Bartke, 1974) and LH receptor content (McNeilly et al, 1979). These effects of FSH on the Leydig cells must be mediated via the Sertoli cells since FSH receptors are found only in the latter cell type (Orth & Christensen 1977). Two secretory products of Sertoli cells, oestradiol (q.v.) and GnRH-like factor (q.v.) can influence Leydig cell function but they both have inhibitory effects. It remains to be seen whether other "Sertoli cell factors" could mediate the effects of FSH on Leydig cells.

3.4 Prolactin

Although the experiments of Woods & Simpson (1961) suggested a synergistic role of prolactin in maintenance of Leydig cell function, this was only confirmed and extended in the last decade with the availability of prolactin RIA and receptor-binding techniques. The presence of prolactin receptors in rat Leydig cells has been confirmed (Aragona et al, 1977; Charreau et al,

1977). Evidence for a positive role of prolactin in control of Leydig cell function came from three animal experimental models: (1) hypophysectomized rats, (2) hereditary dwarf mice deficient in prolactin and growth hormone and (3) light-deprived and testicular regressed golden hamsters. All three models are characterized by testicular atrophy, low circulating prolactin, testosterone and gonadotrophins, and loss of Leydig cell LH receptors. Administration of prolactin increased the LH receptor concentration and restored testicular steroidogenesis in the presence of LH (Bartke et al, 1978; Purvis & Hansson, 1978 for review). In addition, Bartke (1976) suggested that prolactin may augment the effect of LH by stimulating an accumulation of esterified cholesterol and the direct activation of 3β-hydroxysteroid dehydrogenase and 17β-hydroxysteroid dehydrogenase. In humans, nocturnal or pharmacologically-induced prolactin increase had been correlated with increases in testosterone concentration (Rubin et al, 1975, 1976). Pathologically elevated prolactin levels, usually due to pituitary adenomata, is associated with impotence, subnormal circulating testosterone and possibly impaired fertility (Thorner et al, 1977; Carter et al, 1978; Franks et al, 1978). Thus in spite of the convincing evidence in small mammals, the physiological role of prolactin in the human male is uncertain and it may be that prolactin possesses both positive and negative effects on Leydig cell functions depending on its circulating concentration.

3.5 Growth hormone

The effect of growth hormone on gonadal function was again first recognized by Woods & Simpson (1961). Although the specific binding of growth hormone to testicular receptors had not been studied, it was possible to show that growth hormone prevented the loss of LH receptors after hypophysectomy in immature (Bambino et al, 1980) and adult (Zipf et al, 1978) rats. Growth hormone is also able to increase testicular responsiveness to LH in immature hypophysectomized rats (Swerdloff & Odell, 1977). Interpretation of some of these findings are complicated by the overlap in biological effects and receptor sites (human growth hormone is lactogenic) between growth regulation of Leydig cell steroidogenesis.

3.6 Oestrogens

The suppressive effect of exogenous oestrogens on testicular functions was clearly demonstrated by Moore & Price (1932). The conclusion of these workers that oestrogens induce testicular atrophy by suppression of gonadotrophins rather than by direct action on the testes has remained unchallenged until recently. The existence of high affinity cytoplasmic and nuclear receptor sites for oestradiol in rat interstitial cells (Brinkman et al, 1972; Mulder et al, 1974) and the confirmation of direct inhibitory effects, independent of gonadotrophins, of physiological amounts of oestrogens on specific steps in Leydig cell steroidogenesis (reviewed by Moger, 1980) is strong evidence in favour of a negative regulatory role

of intratesticular oestrogens. The cellular site of oestradiol synthesis in the testis has been discussed already (2.5.1). Whether the source of oestrogen is from the Leydig or Sertoli cells is obviously important in formulating fundamental concepts such as an ultra short-loop feedback mechanism or a functional link between the seminiferous tubular and the interstitial compartments of the testis. This is at present unresolved.

CHAPTER 4

TESTICULAR STEROID SECRETION

Examination of synthetic pathways is mainly a qualitative study of the capacity to synthesize hormones according to enzymic capabilities. To understand the quantitative aspects of testicular steroidogenesis, it is necessary to consider the secretion of hormones into the systemic circulation, the rate of secretion, the interconversion and metabolism in the circulation and in target tissues. The aim of these in vivo studies of the dynamics of testicular steroid secretion and interconversion is to assess the biologically effective androgen concentration to which target organs are being exposed. This is a necessary preliminary to the understanding of the mechanism of androgen action at the target cell and receptor levels.

Early studies of testicular androgen secretion had to be based on indirect methods which estimated the urinary metabolites of the hormones under consideration (Vande Wiele et al, 1963; Tait & Burstein, 1964). These were unsatisfactory for several reasons, amongst which the most important were the non-specificity and the multiple anatomical compartmentalization in the production of the urinary metabolites (Korenman & Lipsett, 1964; Tait & Horton, 1964). With the availability of satisfactory methods of measuring physiological concentrations of testicular steroids in blood, it became possible to study the dynamics of testicular steroid secretion in the

circulation combined with radioisotope tracer techniques. These studies were also instrumental in establishing the concept of a prehormone - a substance secreted by endocrine glands which is converted peripherally into a hormone with enhanced or altered biological activity (Baird et al, 1968). This is not unique to gonadal steroids and other prehormones including thyroxine, vitamin D and angiotensin I have since been described.

4.1 Principles of steroid dynamics studies

The principles of these methods were set out in two classic papers (Tait, 1963; Baird et al, 1969a).

4.1.1 Metabolic clearance rate and blood production rate

The plasma concentration of a compound is determined by its rate of entry and rate of removal or clearance from the circulation. The latter occurs in a multitude of organs and tissues where the active hormone is either irreversibly metabolized or excreted. The metabolic clearance rate (MCR) is thus the sum total of clearance rates in individual organs and tissues and is usually defined as the volume of blood which is completely and irreversibly cleared of the hormone per unit time. The quantity of hormone cleared per unit time will be the product MCR x [i] where [i] is the plasma concentration of the hormone during the period of study. Under steady state conditions where plasma hormone concentration remains constant, the rate of production will equal the rate of clearance. This concept was utilized in the indirect measurement of MCR and blood production rate (P_B)

which is defined as the total amount of hormone entering the peripheral circulation from all sources - by direct glandular secretion and/or peripheral conversion of precursors. The entry of the hormone into the circulation can be simulated in vivo by a constant intravenous infusion of an isotopically-labelled hormone at rate (r) while the concentration of the radioactive hormone is measured in blood or plasma at equilibrium or constant concentration (Xc). The MCR is derived by the ratio of these two parameters r/Xc since r = MCR x Xc. If the total non-radioactive concentration of the hormone in the peripheral circulation (i) is also measured, the blood production rate can be obtained - P_B = MCR x i. The validity of blood production rate estimation from MCR is critically dependent on the premise that measurements were obtained under steady state conditions. This, however, is seldom achieved under physiological conditions due to the variable blood production rates resulting from diurnal variation and pulsatile hormone secretion and changing MCRs from the effects of posture. Nonetheless, the effects of diurnal variation and posture can be allowed for in MCR measurements while the above concepts are valid for both constant infusion or single bolus injection. Pulsatile changes in hormone concentrations moreover are no different from constant secretion as far as the total amount of hormone per 24 hours is concerned (Santen & Bardin, 1973). The estimations of blood production rates by steady state dynamic measurements, even in the presence of pulsatile hormone secretion,

should be reasonably accurate.

4.1.2 Protein Binding

The specific high-affinity binding of testosterone, DHT and androstenediol to sex-hormone-binding globulin lowers the hepatic extraction and hence the MCR of these hormones. Specific plasma protein binding is thus an important determinant of MCR - the higher the percentage bound the lower the MCR and vice versa. This is well illustrated by the difference in the MCR of testosterone in males and females and also after acute and chronic testosterone administration (Vermeulen et al, 1969; Southren et al, 1968). It follows also that specific plasma protein binding also determines the unbound biologically-active fraction of the circulating hormone. Hence if other variables such as hepatic blood flow or extrahepatic clearance remain constant, the blood production rate (MCR x [i]) rather than the total plasma concentration of the hormone is better correlated with the biologically active moiety.

4.1.3 Transfer constants

Measurement of blood production from MCR in blood eliminates the necessity of employing mathematical model compartments in the calculation or urinary production rate (Vande Wiele et al, 1963; Tait & Horton, 1966). The P_B is the total amount of hormone entering the circulation from whatever source or compartment and equals the secretion rate if the hormone is secreted exclusively by the endocrine gland (for example, cortisol and aldosterone).

In contrast, testosterone, androstenedione and oestradiol are also produced in extraglandular compartments by peripheral conversion of precursors. If these peripherally-produced hormones reach the circulation before being metabolized, they will contribute to the total blood production. Under these circumstances, $P_B^{Pro} = S_B^{Pro} + S_B^{Pre} \times [P]_{BB}^{Pre-Pro}$ where S is secretion rate of precursor (Pre) or product (Pro) and $[P]_{BB}^{Pre-Pro}$ the transfer constant, is the fraction of precursor converted to product entering the peripheral circulation. The latter is obtained by the constant infusion of radiolabelled precursor hormone(s) until steady state, when the radioactive hormone concentration (X) of both precursor and product are determined. The fractional conversion from precursor to product is obtained by $MCR^{Pro}/MCR^{Pre} \times X^{Pro}/X^{Pre}$. Knowing the total blood production rate and the amount yielded by peripheral conversion will allow the glandular secretion rate to be assessed indirectly.

4.1.4 Steroid prehormones

Although the indirect measurement of secretion rates of testicular steroids have been largely superceded by the direct measurement of testicular venous effluent concentrations (Kelch et al, 1972; Baird et al, 1973; Weinstein et al, 1974), the study of dynamics of hormone secretion and interconversion introduced an important concept in the control of steroid hormone function. Pre-hormones, secreted by the gonads, are converted peripherally into other active metabolites. This

represents a separate mechanism of control by peripheral target or non-target tissues, independent of the control normally operative on the prime source of active hormone. The significance of prehormone conversion may reside in the enhancement of local specificity or potency of hormone action (Baird et al, 1968) or the alteration of local or systemic hormone action by changes in body composition (Siiteri & MacDonald, 1973). Peripheral conversion accounts for the source of testosterone (from androstenedione) in prepubertal males, hypogonadal males and adult females, and oestrone and oestradiol (from androstenedione and testosterone) in children, adult males, postmenopausal and anovulatory females.

4.2 Androgen production in adult males (Table4.1)

Applying the principles and methods discussed in the previous section, it has been possible to establish the production and secretion rates of most steroids in the biosynthetic sequence of testicular androgens. Although it has been postulated that the secretion of some intermediates is an epiphenomenon secondary to a leaky endocrine gland (Short, 1960), the substantial quantity of some steroids secreted by the testis allows the reasonable speculation that they may subserve some biological roles, perhaps as prehormones. This has given rise to the approach of studying the "profile" of testicular steroids as an overall index of testicular steroidogenic capacity and also of peripheral androgenic function.

Steroid	Daily Blood Production	Testicular Secretion	Peripheral Conversion	Adrenal Secretion	References
Testosterone	7 mg	>95%	2.5%	-	499, 880, 364, 353
Dihydrotestosterone	280-390 µg	20%	75-80%	-	260, 656, 379, 530
5α-Androstane-3α,17β-diol	200 µg	?30%	70%	-	430, 431, 79, 585
Androstenedione	1-2 mg	45%	15%	40%	279, 936, 353, 294
Dehydroepiandrosterone	7-9 mg	<10%	15%	80%	294, 458, 459, 959
DHEA-sulphate	10 mg	-	30%	70%	294, 458, 459, 959, 354
Androstenediol	1 mg	60%	10%	30%	80, 200
17α hydroxyprogesterone	2 mg	90%	<10%	-	852, 257, 871
17α hydroxypregnenolone	2 mg	30%	-	70%	71
Oestrone	70 µg	<10%	50%	<10%	See text
Oestradiol	40 µg	25%	75%	-	See text

Table 4.1

Blood production and the source of sex steroid hormones in adult males

4.2.1 Testosterone

In the adult male, testosterone is the most important androgen quantitatively and qualitatively. Testicular vein concentration of testosterone varied between 10-100 µg/dl while the secretion rate is about 7 mg per day with a wide range of 3.5-10. The major precursors are androstenedione and dehydroepiandrosterone which together contribute under 2.5% of blood production. This implies that testosterone can generally be regarded as exclusively secreted by the testis. Thus, testosterone concentration in plasma is one of the best indices of testicular secretory function.

4.2.2 Dihydrotestosterone

The concentration of dihydrotestosterone in the peripheral circulation is relatively low - one tenth to one fifteenth of testosterone. The blood production rate is 280-390 µg of which 75-80% are derived from peripheral conversion of testosterone and androstenedione. This correlated well with spermatic vein concentration measurements estimating testicular secretion to contribute 20% of blood production. The low peripheral concentrations of DHT and its extremely high affinity to SHBG is compatible with the concept that it is principally a target cell hormone (q.v.).

4.2.3 5α-Androstane-3α,17β diol (3α-DIOL)

This 5α-reduced metabolite of testosterone is a potent androgen with peripheral concentrations of 77-190 pg/ml. The blood production is 208 µg/day of which 70%

is converted from testosterone via DHT.

4.2.4 Androstenedione

This immediate precursor of testosterone is found in the peripheral circulation in concentrations between 870-2440 pg/ml. Spermatic venous concentration of this weak androgen confirmed that it is secreted by both the testis and the adrenal in roughly equal quantities. A small fraction (up to 15%) of the blood production is derived from conversion of testosterone and dehydroepiandrosterone in similar proportions. Androstenedione is an important precursor accounting for two thirds of circulating testosterone in prepubertal boys and adult females (Frasier & Horton, 1966). It is also an important substrate for the extraglandular production of oestrogen in both males and females. The testicular secretion of androstenedione varies little with age. Thus in prepubertal boys, testicular androstenedione secretion exceeds testosterone. This is reversed as the testis matures during puberty. The mechanism for this shift in oxido-reduction balance of 17-hydroxysteroid dehydrogenase at puberty is unknown.

4.2.5 Dehydroepiandrosterone (DHEA)

This is the major unconjugated adrenal androgen with under 10% of blood production being secreted by the testis. It is secreted directly or as the sulphated-conjugate. The adrenal vein concentration of DHEA is about fifty times higher than that in the testicular vein. The daily blood production of DHEA is 7-9 mg but 15% of

this is derived from peripheral conversion mainly from the sulphated conjugate.

4.2.6 Androstenediol

This is the Δ_5 precursor of testosterone secreted by both the testis and the adrenal cortex. In adult males, two thirds of the blood production is secreted by the testis while peripheral conversion from DHEA accounts for about 10%. The role of androstenediol as a prehormone is at present uncertain.

4.2.7 17α-hydroxyprogesterone and 17α-hydroxypregnenolone

17α-hydroxyprogesterone is secreted by the testis in amounts which account for 90% of its daily blood production of 2 mg. It is thus a good index of testicular function in normal males. Adrenal suppression has little effect on plasma concentration of 17α-hydroxyprogesterone and it is unlikely that peripheral conversion of 17α-hydroxy-pregnenolone could account for more than 10% of blood production. In physiological concentrations, 17α-hydroxy-progesterone has virtually no androgenic activity. Another notable feature is the extremely marked diurnal variation in plasma concentration of 17α-hydroxy-progesterone with a much greater magnitude than that of testosterone. The specific urinary metabolite of 17α-hydroxyprogesterone is pregnanetriol and the measurement of these two compounds are of diagnostic significance in congenital adrenal hyperplasia with C_{21} hydroxylase defect.

Although 17α-hydroxypregnenolone has a similar blood production rate (2 mg per day) as its Δ_4 counterpart,

only 30-40% is secreted by the testis. The remainder is derived from the adrenals.

4.3 Oestrogen production in males

The capacity of testicular tissues to synthesize oestrogens has been repeatedly demonstrated (for review see Armstrong & Dorrington, 1977). However, the knowledge that exogenously administered androgens can be converted to oestrogens even in the absence of the testes and adrenals (West et al, 1956) raised the possibility that oestrogens in the male could be derived from conversion of secreted androgen precursors rather than by direct secretion.

4.3.1 Oestrone

This is the predominant oestrogen in males or in postmenopausal and anovulatory females. The plasma concentration of oestrone (60-80 pg/ml) is generally found to be one to two times higher than oestradiol. But unlike oestradiol, oestrone shows a significant diurnal variation where the trough levels around 17.00 hours are some 50% of peak levels at 08.00 hours. This obviously complicates the estimation of blood production rates (Baird et al, 1969a). However, even by using the lowest P_B value of oestrone (70 µg/day) there remains a significant fraction of the blood production not accounted for by peripheral conversion of androstenedione (30-40 µg) and oestradiol (6 µg)(Baird et al, 1969a). testicular secretion and adrenal secretion, under 8 µg from each source (Baird et al, 1973; Weinstein et al, 1974; Baird

et al, 1969b). It is therefore likely that another physiological precursor of oestrone is yet to be identified.

4.3.2 Oestradiol

From the substantial concentration difference in spermatic (1-2000 pg/ml) and peripheral (20-30 pg/ml) vein concentrations, it can be estimated that the testis secretes around 10 µg of oestradiol daily (Kelch et al, 1972; Baird et al, 1973; Weinstein et al, 1974). This constitutes one quarter of the oestradiol blood production of 40 µg per day (Longcope et al, 1969) while the remaining three quarters come from the peripheral conversion of testosterone (Baird et al, 1969a).

4.3.3 Significance of oestrogens in the male

While the roles of androgens in the female in the control of sexual hair growth and maintenance of muscle mass are well recognized, the physiological functions of oestrogens in males, if any, are as yet unknown. The fact that peripheral conversion rather than testicular secretion provide the major source of oestrogens in man create further conceptual difficulties. The ease with which oestrogenic effects are manifested when the rate of aromatization is increased and/or when the testosterone/oestradiol ratio is depressed as in hepatic cirrhosis (Vermeulen, 1977; Gordon et al, 1975; Baker et al, 1976), obesity (Siiteri & MacDonald, 1973) and ageing (Aiman et al, 1978) makes it reasonable to speculate that oestrogens may have some as yet undefined physiological

functions.

Receptors for oestrogens in the male have been identified in the testicular Leydig cells, prostate, adrenals, pituitary and hypothalamus of the rat and in the pituitary and hypothalamus of foetal human brain (Davies et al, 1975). Furthermore, local formation of oestrogen has been demonstrated in the diencephalon of the human brain (Naftolin et al, 1975; Ryan et al, 1972). It has been suggested that the effect of androgens on the brain in the control of sexual behaviour are effected via conversion to oestrogens locally (McDonald et al, 1970; Beyer et al, 1973). Oestradiol is an effective inhibitor of gonadotrophin secretion (Kulin & Reiter, 1972; Sherins & Loriaux, 1973; Stewart-Bentley et al, 1974) and pituitary response to GnRH stimulation (Wang et al, 1975) in men. The exact role of oestrogens in relation to androgens in the physiological control of gonadotrophin secretion is unclear (q.v.). The effect of pharmacological doses of oestrogens in raising SHBG concentration is well described (Pearlman et al, 1967). However, whether physiological levels of oestrogens have any effects on androgen function and SHBG binding has not been studied.

4.4 Steroid conjugates

Although steroid conjugates are insignificant precursors for testosterone blood production, the human testis has been shown to secrete them in significant quantities (Laatikainen et al, 1969 & 1971). Metabolic

changes in the steroid nucleus can occur without hydrolysis of the conjugate (Lieberman, 1967). It thus remains a possibility that these compounds are not merely water-soluble end products of metabolism to be eliminated from the body but play a more active physiological role. Virtually all tissues in the body except during foetal life contain steroid sulphatases which should theoretically be capable of releasing free androgens from the circulating conjugates with or without further conversion to more active metabolites. This hypothetical system may allow the expression of androgenic effects in the local environment of a wide variety of tissue sites.

CHAPTER 5

TRANSPORT OF CIRCULATING ANDROGENS

All steroid hormones in the peripheral circulation are bound by plasma proteins to various extents by dissociable non-covalent bonds. Albumin binds non-specifically to steroid hormones with low affinity (Ka $\sim 10^4\ M^{-1}$) but high capacity (plasma concentration :- 5.6×10^{-4} M). In contrast, a family of glycoproteins bind specifically to individual steroids with high affinity (Ka $\sim 10^8\ M^{-1}$) but low capacities (plasma concentrations 10^{-6} - 10^{-8} M).

The existence of a specific binding protein in human plasma for testosterone was first demonstrated by Mercier et al (1966) and shortly afterwards confirmed by Crepy et al (1967). It also binds many other C_{18} or C_{19}-17β-hydroxysteroids e.g. DHT with two to three times greater affinity, androstanediol and androstenediol with similar affinity and oestradiol with 75% of the affinity of testosterone (Vermeulen, 1977). This led to the protein being named the sex hormone binding globulin (SHBG) or testosterone-oestradiol binding globulin (TeBG). Recently, more stringent physiological studies of the binding characteristics of SHBG revealed that oestradiol does not bind to SHBG to any significant extent (Vigerski et al, 1979). This therefore is similar to the weaker androgens androstenedione, DHEA and DHA-sulphate, which are almost exclusively bound to albumen. Testosterone also binds to other plasma proteins such as transcortin

and α_1-acid glycoprotein with low affinities only (Vermeulen et al, 1971; Kerkay & Westphal, 1968).

5.1 Physico-chemical properties of SHBG

SHBG is a β-globulin produced in the liver, with molecular weight of 92,000 (Mercier-Bodard et al, 1979). Because of its instability on purification, SHBG of sufficient purity has not been available in sufficient quantity for detailed physico-chemical studies. Until recently therefore, specific RIA was not feasible and plasma SHBG concentration must be estimated indirectly by means of its binding activity. Since neither the molecular weight and the number of binding sites on each molecule were accurately known, SHBG concentration was expressed in terms of the concentration of steroid binding sites in 10^{-8} M units. However, as the purification procedures have become more satisfactory and specific RIAs have become available, SHBG concentrations in plasma can be directly determined (Mercier-Bodard et al, 1979).

In normal males, 2% of circulating testosterone is unbound, 56% is SHBG-bound, and 38% is albumen-bound as studied by equilibrium dialysis of 1:5 diluted plasma at pH 7.4 and 37°C (Vermeulen, 1977). SHBG concentration and hence the free testosterone concentration is not constant throughout life. While SHBG binding capacity is low in late pregnancy and the newborn (Forest & Bertrand, 1975), this increases by the second postnatal week to three times the adult concentration so that despite the

neonatal surge of testosterone, the free testosterone fraction is only 0.7% giving paradoxically a lower free testosterone concentration than the newborn period. At puberty, SHBG concentration declines while the trend is reversed during senescence (Vermeulen et al, 1972). The concentration of SHBG in normal adult men is about half that of non-pregnant adult females and has the capacity to bind about double the amount of testosterone normally present in the circulation of adult men (Vermeulen et al, 1969; Corvol et al, 1971; Rosenfield, 1971).

Drawing on the analogous evidence from cortisol (Slaunwhite et al, 1962) and progesterone (Burton & Westphal, 1972), it has been postulated that hormone binding to specific proteins renders it metabolically inert and biologically inactive. Thus only the unbound fraction is capable of entering the extravascular and intercellular spaces. Mowszowicz et al (1970) provided supportive evidence in finding that in vitro aromatization of testosterone to oestradiol by rat liver microsomes was inhibited by the addition of SHBG. Lasnitzki & Franklin (1972) showed that the uptake, conversion to DHT and morphological changes induced by testosterone in rat prostate explants could be reduced by male serum and even more so by pregnant female serum.

Although hormones specifically bound (with high affinity) to plasma proteins are metabolically and biologically inactive, non-specific binding to albumen has little effect on hepatic metabolism and splachnic extraction (Baird et al, 1969). This implies that the

degree of specific protein binding is one of the major determinants of metabolic clearance of a hormone. A survey of different sex steroid hormones in males and females confirmed that an inverse relationship between the affinity for SHBG and metabolic clearance rates exist (Southren et al, 1967, 1968; Longcope et al, 1968; Vermeulen et al, 1969; Mahoudeau et al, 1971; Saez et al, 1972). This is best illustrated by the higher MCR of androstenedione than testosterone in males and the lower MCR of testosterone in females. However, the importance of SHBG in terms of bioavailability of the free hormone to target cells must not be overemphasized despite its important effect on MCR. The following equations demonstrate this point:-

$$\text{Unbound } [i] = \text{Total } [i] \times \% \text{ unbound } i$$

$$* \text{ Total } [i] = P_B / \text{MCR}$$

$$\therefore \text{unbound } [i] = \frac{P_B \times \% \text{ unbound } i}{\text{MCR}}$$

A rise in SHBG will reduce the % unbound i but MCR also decreases proportionately. Thus the absolute concentration of unbound hormone will not change unless P_B is altered. It is therefore worth emphasizing that blood production rate (P_B) rather than SHBG binding is the key variable which controls the unbound hormone concentration available to target cells.

* $[i]$ = steroid concentration P_B = blood production

5.2 Biological roles of SHBG

The solubility of testosterone in saline at 37°C is 55 μmol/l which is 1000 times greater than the maximal concentrations found in normal adult males (de Moor et al, 1968). Clearly a simple carrier function (such as transferrin for iron) is untenable for SHBG. The lowering of MCR by SHBG binding prolongs the steroid hormone half-life in the circulation and may thus allow the maintenance of high functional pools of sex hormones with relatively low rates of glandular secretion.

A more fascinating possibility is that the high-affinity SHBG binding in the circulation is part of a specific transport system for sex steroids. This system starts with glandular secretion and ends in the nuclear chromatin receptor sites in target cells. Androgens are conveyed along a privileged channel made up of a series of specifically-binding proteins with increasing affinity for metabolizing enzymes and cytosol and nuclear receptors.

It was suggested even before the existence of SHBG had been suspected that protein binding of plasma steroids serves to buffer their concentrations against wide fluctuations (Daughaday, 1959). Although pulsatile secretion of GnRH is essential to the normal function of the pituitary, this is not necessarily the case in LH control of testicular functions. Unlike the subprimate mammals, where a close temporal relationship between plasma peaks of LH and testosterone exists, this has been difficult to demonstrate in man (Baker et al, 1975; de

Kretser et al, 1977). The unpredictable or delayed testosterone rise after endogenous or exogenous LH stimulation may be the result of the dampening effect of a large reserve pool of protein-bound testosterone. However, SHBG binding of testosterone in other species, e.g. the ram, albeit of lower affinity than humans, does not seem to dampen the acute testosterone rise in response to pulsatile LH stimulation (Sandford et al, 1974). Thus plasma protein binding does not invariably abolish fluctuations in plasma concentrations of hormones. Another possible role for a high affinity, low capacity binding protein such as SHBG may be to maintain a minimal basal concentration of testosterone while still making higher concentrations of the steroid available to the target tissues when the secretion rate is greatly increased for a short period of time (Tait & Burstein, 1964).

A specific physiological role for SHBG cannot at present be readily defined. The fact that SHBG is absent in some species, e.g. rat, boar (Corvol & Bardin, 1973) implies that this protein is not essential for normal androgen transport and action in all species.

5.3 Effect of other hormones on SHBG concentration

Androgens administered to women will lower the SHBG binding capacity (Vermeulen et al, 1969) while oestrogens given to males have the opposite effect (Pearlman et al, 1967; Migeon et al, 1968; Vermeulen et al, 1969). During pregnancy, SHBG binding increases some

five times with the sharpest rise being in the first trimester (Vermeulen, 1977). Medroxyprogesterone acetate and other synthetic androgens decrease SHBG binding in plasma of both sexes (Forest & Bertrand, 1972; Makhzangy et al, 1979). Corticosteroids in high doses also decrease SHBG binding (Vermeulen et al, 1969). Thyroid hormone excess induces a significant rise in SHBG probably as a result of increased hepatic synthesis (Chopra & Tulchilsky, 1974; Olivio et al, 1970; Vermeulen, 1977).

CHAPTER 6

MECHANISM OF ACTION OF ANDROGENS

It is interesting that relatively small amounts of chemically quite simple androgenic steroid molecules can produce such highly specific and widely varying biological effects in a diversity of tissues - consider androgen-controlled processes such as sexual differentiation, pubertal growth, liver enzyme and foetal haemoglobin synthesis and the activation of sexual behaviour. Any proposed mechanism(s) of action of androgens must therefore seek to explain this specificity and diversity of action simultaneously.

6.1 Basic model of molecular mechanism of androgen action

In the late 1960s, studies of the intracellular metabolism of radioactive-labelled testosterone revealed that the injected androgen was rapidly and irreversibly metabolized by the rat ventral prostate to DHT, in which form it is specifically retained in the cell nuclei as the principal intracellular androgen (Bruchovsky & Wilson, 1968; Anderson & Liao, 1968). Shortly afterwards, it was demonstrated that soluble cell-free fractions from rat ventral prostates contained receptor proteins that specifically bind DHT with ten-fold greater affinity than testosterone (Fang et al, 1969; Mainwaring, 1969). DHT is 2.5 times more potent than testosterone in many bio-assays (Dorfman & Shipley, 1956; Bardin & Mahoudeau, 1970). One of the earliest biochemical responses of

androgen stimulation - nuclear RNA synthesis - could not be induced by testosterone added to prostatic cell nuclei in vitro (Liao et al, 1965). These observations suggested that DHT might be the locally active metabolite of testosterone which binds to specific receptors and initiates nuclear mechanisms relevant to the cellular androgen response. In subsequent years, most androgen responsive tissues have been shown to possess receptor proteins capable of binding DHT, testosterone and other 5α- and 5β-reduced metabolites of testosterone as well as oestradiol (Verhoeven et al, 1975; Attramadal et al, 1976; Bardin & Catterall, 1981). Thus it appears that specificity of androgen action is achieved by the high affinity hormone-receptor interaction while diversity is conferred by the local conversion of the parent androgen molecule to different active metabolites and by the activation of the genetic machineries of different target cells each with their respective mode of response. Testosterone is therefore considered as both a hormone and a prehormone, acting as a precursor for biologically active metabolites, the most important of which include DHT, oestradiol and 5α-androstanediol (Mainwaring, 1980). Other less well-defined local metabolites of testosterone may occur in classical target tissues as well as tissues previously not thought to be androgen-responsive (Rosness & Eik-Nes, 1977). Implicit in this broadened concept of androgen action is the belief that the diversity of androgen action is introduced by the evolution of different local metabolizing enzymes in target tissues

and modification of the structure of nuclear chromatin rather than by different circulating androgens which will put an unreasonable demand on the synthesizing capabilities of the Leydig cells. In other words, the androgenic response is organ or tissue-specific rather than steroid-specific and our understanding of the mechanism of androgen action should be advanced by focusing attention on the molecular mechanisms of androgen metabolism, receptor binding and nuclear chromatin activation in diverse tissues.

6.2 Receptors binding and activation

At least two forms of receptor proteins for DHT have been identified in the rat ventral prostate; the α protein and β protein forming complexes I and II respectively with DHT. Only the latter is translocated into the nucleus so that only β protein is regarded as the biologically significant androgen receptor (Liao et al, 1973a). Unlike SHBG, β protein has little affinity for other steroids except androgens to which it is bound with an affinity one to two orders of magnitude higher than SHBG. This step up of binding affinity between plasma and intracellular binding proteins may be an important part of the specific transport system for the transfer of androgens from the vascular to the tissue compartments against concentration gradients.

Receptor-DHT binding results in the formation of an activated receptor-hormone complex which is temperature dependent and associated with changes in the isoelectric point and sedimentation constant (Mainwaring & Irving,

1973; Liao, 1975). This activated complex can be translocated into the nucleus and is capable of binding to nuclear chromatin. Attempts at purification of this receptor-DHT complex have so far met with little success (Mainwaring, 1977). As in oestrogen receptors, it is generally accepted that the duration and extent of retention of the receptor-DHT complex in the nucleus is crucial to the magnitude of androgen-induced responses (Katzenellenbogen et al, 1979). It has also been suggested that specialized structural elements exist which facilitate the nuclear retention of receptor-DHT complexes. The nature of this acceptor site is unknown but DNA associated with non-histone protein (Mainwaring & Peterkin, 1971) and nuclear associated RNA (Liao et al, 1973b) have been postulated as likely candidates. In general target tissues such as rat ventral prostate have a higher capacity to retain the activated receptor-DHT complex than non-target tissues (Fang & Liao, 1971; Mainwaring & Peterkin, 1971) and nuclear binding of androgens, other than 5α Diols which have an exclusively cytoplasmic site of action, is the hallmark of their response.

6.3 Biochemical responses

The observations that cyproterone acetate, an anti-androgen that inhibits receptor binding, can abolish the early increase in nuclear RNA synthesis (Mainwaring et al, 1974) and that cytosol preparations of DHT-receptor complex can enhance RNA synthesis in cell free prostatic

nuclear preparations (Davies & Griffiths, 1974) suggest that chromatin binding of the activated receptors acts as a trigger for transcription of genetic information stored in the DNA of the genome. This initiates a complex and poorly understood sequence of molecular events resulting in increased synthesis of ribosomal RNA for general protein synthesis, messenger RNA to direct specific amino acid synthesis and DNA necessary for cellular division (for review see Chan & O'Malley, 1976).

6.4 Other possible mechanisms of androgen action

The vast majority of data on androgen action was derived from one experimental model - the rat ventral prostate. There is thus a real possibility of over-playing the importance of the DHT receptor binding mechanism to the exclusion of other possibilities. The related question as to whether testosterone functions purely as a prehormone or can initiate cellular response itself without further conversion also arises. Two important target organs, adult seminiferous tubules (Hansson et al, 1975) and skeletal muscles (Gustafsson & Pousette, 1975) have minimal 5α-reductase activity and display testosterone binding only. DHT cannot restore normal male sexual behaviour in castrated rodents which respond to testosterone or oestrogens (Davidson, 1972). In humans, intrauterine differentiation of Wolffian duct-derived structures - seminal vesicles, epididymis and vas deferens are testosterone dependent (Imperato-McGinley & Peterson, 1976) although in post-natal life, these

structures respond preferentially to DHT. It should be obvious that DHT is by no means the universal locally-active androgen and that requirements of target cell responses may vary at different stages of development. Nevertheless, the basic model discussed above remains unchallenged by other known or postulated molecular pathways. Androgens may activate cyclic AMP, modify lysosomal and plasma membrane functions or bind with 5β steroid receptors of target cells but none of these mechanisms interact with the classical androgen receptor or the nucleus. Examples of these "non-classical" androgen actions are encountered in certain enzyme synthesis in the prostate and protein synthesis in the liver and bone marrow (Bardin & Catterall, 1981). It is evident that the action of androgens in some tissues may be mediated by a variety of independent mechanisms which have yet to be defined.

CHAPTER 7

BIOLOGICAL EFFECTS OF ANDROGENS

Although male sex hormones are not essential for survival of the individual, they are however critical to the satisfactory propagation of the species. This is achieved not only through the effects of androgens on the reproductive tract but virtually all tissues in the body are influenced by the action of sex steroids. In this chapter, those androgen-dependent functions with the greatest theoretical interest and/or clinical implications are discussed. The effects of androgens on spermatogenesis and the physical changes at puberty induced by androgens are mentioned but will be dealt with in greater detail in subsequent sections.

7.1 Sexual differentiation

Sexual differentiation is the result of the translation of genetic into gonadal and phenotypic sex through an orderly hierarchy or regulatory systems which originate in the testis-determining gene in the Y chromosome. The experiments of Jost (1970) established that male development is induced in the embryo only in the presence of specific hormonal signals from the foetal testis. If an ovary develops or if no gonad is present, female phenotype differentiation will result. Thus in the presence of a Y chromosome and the normally functioning testis-determining gene in the pericentric region, the male-specific diffusable cell-surface antigen,

HY antigen, diverts the inherent inclination of the indifferent embryonic gonad from developing into an ovary and forms a testis instead (Wachtel et al, 1975; Ohno, 1976). This occurs at around eight weeks of foetal life. Once the foetal testis is formed, it is the secreted testicular hormones which magnify and perpetuate the genetic message coded in the HY gene of the Y chromosome and elicit the development of the male internal and external genitalia (Wilson et al, 1980). Normal function of the testis and testicular hormones also depend on subsidiary regulatory mechanisms and their structural genes which may reside in the X chromosome or the autosomes (Haseltine & Ohno, 1981).

Differentiation of the reproductive tract occurs during the first trimester after the formation of the gonads. In the male, the Müllerian duct regresses under the ipsilateral action of the glycoprotein hormone, Müllerian Inhibiting Hormone, produced by the foetal Sertoli cells (Josso et al, 1977). Development of the wolffian duct into male internal genital tract (epididymis, vas deferens, seminal vesicles and ejaculatory duct) takes place under the influence of testosterone itself since little 5α-reductase activity is detectable. However, the urogenital sinus, the genital swelling, genital fold and genital tubercle do have an active 5α-reductase and the preferential binding of 5α-dihydrotestosterone directs the embryonic differentiation of the prostate, scrotum, urethra and penis (Wilson et al, 1980). Two aspects of intrauterine sexual development have been

relatively neglected and remain poorly understood at present. Testicular descent takes place in the second and third trimester. The physical and hormonal factors involved are not clearly defined; gonadotrophins, testosterone and Müllerian inhibiting hormone have all been implicated. The early development of the seminiferous tubules are also ill-understood. Regions in the Yq arm of the Y chromosome can influence the formation and development of the spermatogonia (Tiepolo & Zuffardi, 1976) while the X chromosome must be inactivated prior to meiosis (Cattanach et al, 1971). The exact relationship between the genetic regulation and the interaction with the testicular somatic elements in the early development of germ cells constitutes an important area for future research.

Our understanding of the hormonal mechanisms of sexual differentiation has been greatly aided by certain mutant forms in man and animals. Two foremost examples are the testicular feminization syndrome and patients with 5α-reductase deficiency. In the former, the X-linked gene controlling the cytosol androgen receptor protein is defective. Even though the embryonic testis secretes normal amounts of testosterone, none of the cells can respond as the androgen receptor is absent or abnormal. Consequently, the individuals have a female phenotype but the internal genitalia is unformed and they are infertile (Morris, 1953; Morris & Mahesh, 1963; Lyon & Hawkes, 1970). In the second group of patients, an autosomal recessive disorder of 5α-reductase deficiency or malfunction gives

rise to a distinct syndrome of abnormal sexual differentiation - pseudovaginal permeoscrotal hypospadius (Walsh et al, 1974; Imperato-McGinley, 1974). Since the testosterone level and androgen receptor are normal, these individuals have an essentially male phenotype and wolffian duct structures are formed. However, the external genitalia are predominantly female with a blind vagina and an enlarged clitoris. At the time of puberty, increased testosterone secretion stimulates the postnatal growth of the phallus and skeletal musculature as well as the onset of spermatogenesis. These experiments of nature provide excellent models in which to examine the theories of sexual differentiation and it can be said that the present concepts of genetic and hormonal mechanisms of sexual differentiation have been largely substantiated.

7.2 Secondary sexual characteristics and external genitalia

Differentiation of the accessory glands (seminal vesicles, prostate, epididymis, periurethral glands) and the external genitalia are completed in the first trimester of foetal development. Although some growth of the external genitalia in the mid and third trimester occurs, further growth and functional maturation of the accessory glands are deferred until the time of secondary sexual development when circulating androgen levels rise to the adult range. The effects of prepubertal castration and the response of the prostate and seminal

vesicles to exogenous androgen replacement have been well recognized and indeed provided the basis of one of the first bioassay systems available.

Although this classical mechanism of cytosol receptor protein binding, nuclear translocation and chromatin activation applies to the whole genital tract, the functional response to androgens of different accessory glands shows a remarkable degree of tissue specificity. Thus in both the seminal vesicles and prostate, approximately 40% of total protein synthesized are secreted into the urogenital tract, but each gland produces distinct and characteristic secretory products (Mann, 1975). Tissue specificity of androgen action is further illustrated by the diverse changes in body functions in puberty characterized by increased size of external genitalia, pubic and body hair growth, enlargement of the laryngeal cartilage, increased sebum secretion, accelerated growth rate and maturation of the skeletal system. Observations in patients with 5α-reductase deficiency indicate that these changes in secondary sexual development, except those in facial and body hair, are mediated by testosterone rather than dihydrotestosterone. This implies that ontogenic changes in the external genitalia involving the 5α-reductase enzyme system exist so that dihydrotestosterone provides the major stimulus for differentiation while testosterone is the critical hormone for postnatal growth and maintenance of adult function.

7.3 Extragenital actions of androgens

Testosterone, unlike oestrogen, has major effects on the growth and development of many tissues other than those of the genital tract (Kochakian, 1975; Bardin & Catterall, 1981) as evidenced by the changes observed during puberty mentioned above. It is thus the principal hormone responsible for sexual dimorphism in non-reproductive tissues and organs. The widespread actions of testosterone (and other androgens) on extragenital organs such as skeletal muscle, bone, liver, kidney and red blood cells are collectively known as anabolic effects to distinguish them from the virilizing actions on the reproductive organs. Prompted by the clinical and farming interests in obtaining exclusively anabolic steroids, many bioassays were established to select appropriate natural and synthetic compounds with non-virilizing anabolic properties. Unfortunately, this has been hampered by the lack of a universal index of androgenicity and the variability of anabolic effects amongst extragenital tissues and amongst different species. It is therefore not surprising that the results of animal bioassays are often not borne out in clinical practice. These observations reinforce the view that androgenic actions are organ-specific rather than steroid-specific so that it is doubtful whether androgens can be completely separated into anabolic and virilizing steroids.

In recent years, significant advances have been made in elucidating the mechanism of androgen action on non-

genital tissues. These studies not only revealed novel molecular mechanisms of androgen action but also provided excellent models for the study of genetical control of hormonal function. Some of these works are briefly mentioned below.

7.3.1 Skeletal muscle

The myotrophic effect of testosterone is the basis for sexual dimorphism in muscle mass and body weight. This is reflected in the greater retention of dietary nitrogen in the male and constituted one of the classical clinical tests for the anabolic effects of nitrogen (Kochakian, 1975). Testosterone rather than dihydrotestosterone is believed to be the active androgen in skeletal and cardiac muscles where the presence of appropriate receptors have been recently demonstrated (Dionne et al, 1979; McGill et al, 1980).

7.3.2 Liver

Testosterone and to a lesser extent oestrogen regulate the concentration and activities of sexual steroid-hydroxylases and microsomal enzymes important in drug metabolism. Some of these enzyme activities are dependent only on a brief exposure to androgens at birth - an imprinting process analogous to that observed in the central nervous system (Gustafsson & Stenberg, 1974; Gustafsson et al, 1980). Other specific androgen-dependent, liver proteins such as α_{2U}-globulin in rat serum and major urinary proteins in mice urine have been characterized (Roy & Neuhaus, 1967; Roy, 1973). The

mechanism of androgen control of hepatic protein synthesis is through mRNA translation (Kurtz & Feigelson, 1977) but not all the actions of testosterone on the liver are mediated by the classical androgen receptor.

7.3.3 Kidney

Sexual dimorphism of the kidney exists in many species but the most studied is the mouse where cells of the Bowman's capsule and proximal convoluted tubules are significantly larger in males than females (Dunn, 1949; Mills et al, 1979) and specific protein synthesis - β glucuronidase, has been extensively characterized (Paigen et al, 1975). At least six genes are known to regulate this renal hydrolase enzyme, each controlling an individual aspect such as synthesis, molecular forms, storage, intracellular location and inducibility by androgen. These structural and regulatory gene loci are activated by the steroid-receptor complex in the nucleus but the mechanism of this activation is unknown.

7.3.4 Red blood cell

Haemoglobin synthesis in red blood cells is stimulated by two independent processes. The renal secretion of erythropoietin is increased by androgens via cytosol receptor binding (Congote & Solomon, 1975). In contrast, 5β-androgens and 5β-progestagen metabolites directly stimulate the pluripotential stem cells of the bone marrow; this effect is mediated by specific 5β-steroid receptors (Spooner & Mainwaring, 1973).

7.4 Spermatogenesis

Although the biological effects of testosterone are most dramatically illustrated in the development of primary and secondary sexual characteristics and the functional maturation of accessory sex organs, it is the seminiferous tubules within the testis which constitute the single most important androgen-dependent target tissue. Teleologically, it seems expedient that the development of these specialized androgen-dependent cells carrying information from one generation to the next should be guaranteed the earliest and highest availability of androgens by strategically siting the seminiferous tubules immediately adjacent to the source of hormone production. Intratesticular concentration of testosterone is fifty to one hundred times higher than that in the circulation (Steinberger et al, 1974) and there is indirect evidence that these extremely high levels of testosterone may be essential for the development and maintenance of spermatogenesis (Dvoskin, 1944; Ahmad, 1973; Steinberger et al, 1973). The hormonal control of spermatogenesis is dealt with in greater detail in the following chapter.

CHAPTER 8

REGULATION OF SEMINIFEROUS TUBULE FUNCTION

8.1 Spermatogenesis

Spermatogenesis is a continuous and strictly coordinated cytological process in the seminiferous tubules of the adult testis. In the seminiferous epithelium, the immature spermatogonia proliferate by mitotic divisions to form spermatocytes while a minority renew themselves to maintain their own numbers (Clermont, 1966). The diploid spermatocytes go through meiotic reduction divisions to form haploid spermatids whose cell structures become intricately modified to yield the highly differentiated spermatozoa with inherent capacities for motility and fertilization. Morphological studies of the testis established the presence of specific cellular associations in the germinal epithelium made up of fixed compositions of various germ cells at different stages of development (Clermont, 1963). The kinetic basis of the cellular associations was revealed by the precise and fixed intervals separating each step or cellular divisions in spermatogenesis with the use of [^{3}H] thymidine labelling (Heller & Clermont, 1964). The classification of these cellular associations into specific stages was achieved by the characteristic appearance of the acrosome after Periodic-Acid-Schiff haematoxylin staining of the spermatids at different phases of development (Leblond & Clermont, 1952). Thus a complete cycle of the seminiferous epithelium could be

defined as the series of changes in a given area of the seminiferous tubule between two successive appearances of the same developmental stage. The number of stages constituting a cycle is characteristic for each species and in man there are six recognisable stages forming one cycle of seminiferous epithelium lasting approximately sixteen days. The entire course of spermatogenesis in man occupied four to five cycles or approximately seventy days. This interval of time required for the spermatogonia to develop into a spermatozoa is a biological constant characteristic for any one species and cannot be altered by any stimulating or inhibiting factors (Clermont, 1972). This understanding of the dynamics of spermatogenesis has allowed the application to quantitative evaluation of changes in the seminiferous epithelium under various experimental and clinical situations.

While the morphological aspects of the cytological processes taking place during spermatogenesis have been extensively studied, many fundamental issues remain to be tackled. The molecular aspects of these structural modifications, the role of the Sertoli cells, the action of trophic hormones and the interactions between Sertoli, Leydig and germ cells are but some of the areas that require systematic studies.

8.2 Hormonal requirements of spermatogenesis

The classical experiments of Smith & Engle (1927) in hypophysectomized rodents clearly established the

importance of pituitary glandular secretions in spermatogenesis. The dual control of testicular function by individual gonadotrophins, LH for Leydig cells and FSH for spermatogenesis was proposed by Greep et al (1936). The functional relationship between LH and Leydig cell steroidogenesis has since been amply confirmed (see Chapter 4). The role of FSH, however, in spermatogenesis has been disputed. The major objection was the finding that androgens alone can maintain spermatogenesis in hypophysectomized animals (Walsh et al, 1934; Nelson & Merckel, 1938). Other studies claimed that a synergistic action between LH and FSH is necessary for normal spermatogenesis (Woods & Simpson, 1961; Lostroh, 1963). These studies suffered from the lack of quantitative analysis of spermatogenesis, incomplete hypophysectomy and the use of impure gonadotrophin preparations. Their conclusions that FSH may not be required for spermatogenesis must therefore be regarded with some suspicion. The work of Steinberger and colleagues (Steinberger & Duckett, 1967; Steinberger & Steinberger, 1969) using oestrogens to suppress gonadotrophins in newborn rats and in vitro studies on cultures of testicular explants introduced the concept that initiation or restoration may have different hormonal requirements from the maintenance of spermatogenesis and that specific steps in spermatogenesis may also have different hormonal requirements (Steinberger, 1971).

A more specific and selective approach using passive immunization by monospecific antisera against LH or FSH

has been used to study this problem. In rats, FSH and LH or testosterone are necessary to initiate spermatogenesis in immature males (Chemes et al, 1979) while adults require only testosterone to maintain spermatogenesis (Dym, 1979). In contrast, testosterone alone was ineffective in maintaining spermatogenesis in the adult ram despite normal levels of testosterone and dihydrotestosterone within the testes (Monet-Kunz et al, 1976). Both LH and FSH in the form of human chorionic gonadotrophin (hCG) and human menopausal gonadotrophin (hMG) are required (Courot et al, 1979). In adult Bonnet and Rhesus monkeys, FSH was required to maintain quantitatively normal spermatogenesis (Murty et al, 1979; Wickings et al, 1980). In man, both hMG and hCG are needed to initiate or restore spermatogenesis in hypogonadotrophic or hypophysectomized patients (Mancini et al, 1971). These studies clearly indicated that FSH does play an important part in spermatogenesis although there are important species differences in the specific hormonal requirements at different stages of development.

The positive influence of testosterone on spermatogenesis in gonadotrophin deprived animals contrasts with its effect in intact males where atrophy of the seminiferous tubules is the usual result unless pharmacological doses are employed (Moore & Price, 1932; Ludwig, 1950). This differential effect of varying doses of testosterone is probably due to gonadotrophin suppression by exogenous testosterone with very high doses being able to maintain normal spermatogenesis by producing high intratesticular

testosterone concentration in spermatogenesis was well illustrated in the prepubertal testis of a patient with a functioning Leydig cell tumour around which spermatogenesis was stimulated beyond meiosis while the contralateral testis atrophied (Steinberger et al, 1973). The intratesticular testosterone concentration in a small number of men was around 56 µg/dl which was fifty to one hundred times that found in peripheral plasma (Steinberger et al, 1974).

8.3 Sertoli cells

These tall columnar somatic cells with extensive cytoplasm enveloping the developing germ cells are traditionally regarded as the supporting or nurse cells in the seminiferous tubules. Tight junctions between adjacent Sertoli cells form the anatomical basis of the blood testis barrier dividing the seminiferous tubule into two function units: the basal compartment in direct communication with the interstitial space and the adluminal compartment which provides a secluded and avascular environment in which the leptotene primary spermatocytes, secondary spermatocytes and spermatids develop (Setchell & Waites, 1975; Setchell, 1980b; Dym & Cavicchia, 1978) (Fig 8.1 & 8.2). The Sertoli cells which straddle both these functional compartments secrete large amounts of hyperosmolar tubular fluid rich in potassium, inositol, aspartates and glutamates creating a highly specialized microenvironment in the adluminal compartment believed to be important for the completion of germ cell meiosis and

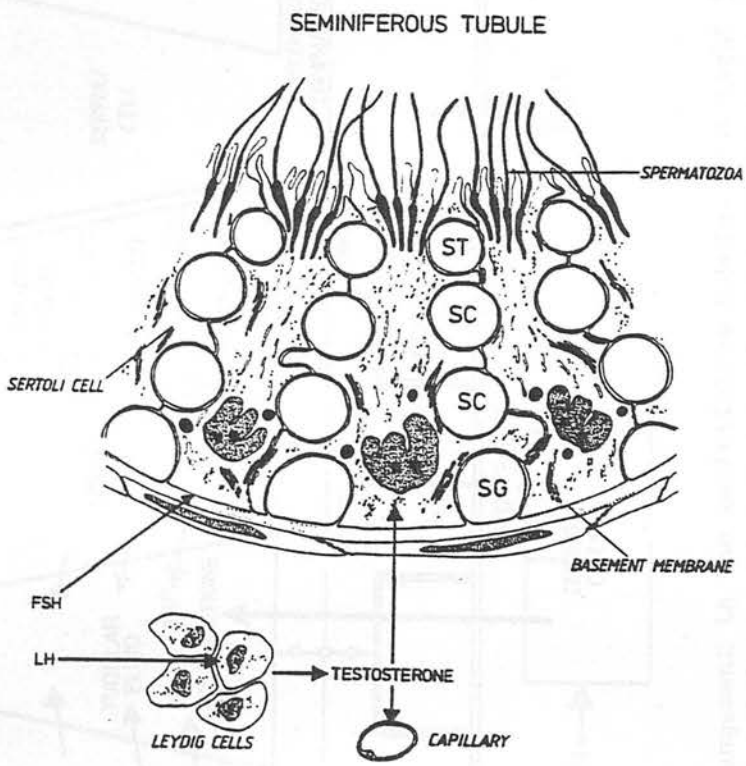


Fig 8.1

Diagrammatic representation of the cellular composition and structural relationship between different cell types in the testis. SG: spermatogonia; SC: spermatocytes; ST: spermatids (from Dorrington, 1980).

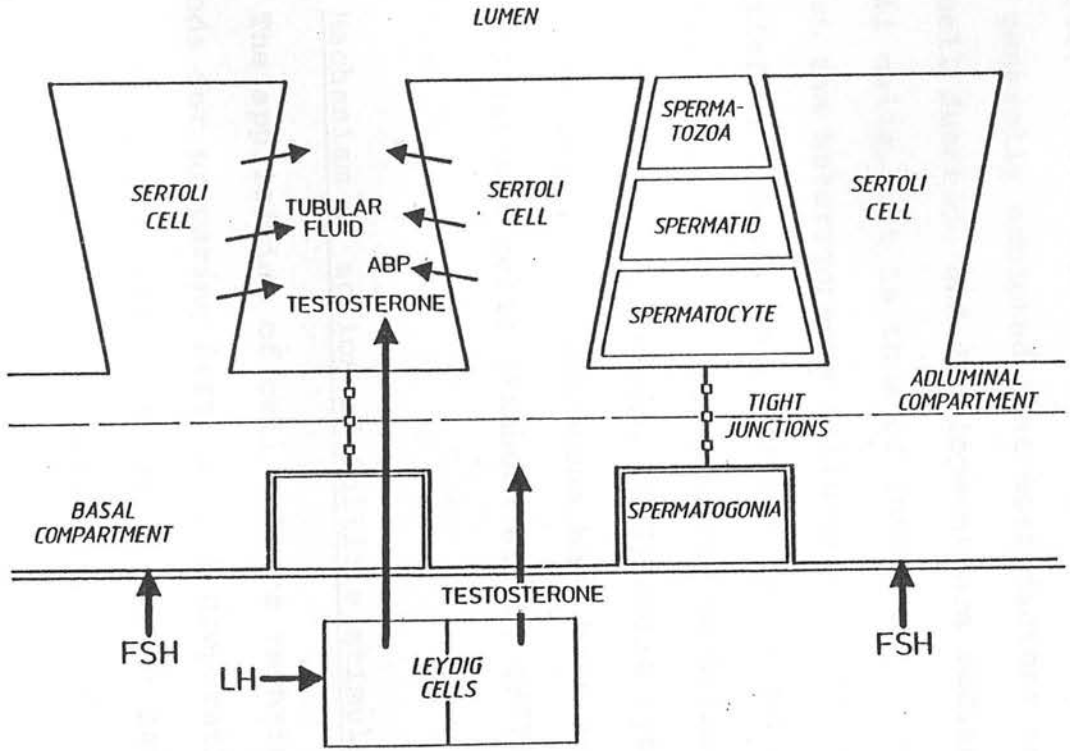


Fig 8.2

Schematic view of the cellular arrangement in the seminiferous tubule. The tight junctions between adjacent Sertoli cells separate the tubule into basal and adluminal compartments. The various postulated modes of hormonal action are indicated. ABP: androgen binding protein. (Adapted from Dorrington, 1980.)

spermiogenesis (Fritz, 1978). Besides the elaboration of tubular fluid, an array of other functions have been ascribed to the Sertoli cells including protein synthesis, phagocytosis of degenerating germ cells, release of mature spermatozoa, movement of germ cells from periphery towards the lumen and metabolism of steroid hormones (Fawcett, 1975). By virtue of their strategic location, it is generally accepted that most factors influencing germ cell function and development are mediated via the Sertoli cells. It is thus of interest to note that amongst the heterogeneous cellular elements of the seminiferous tubule, only Sertoli cells and spermatogonia have been shown to possess specific membrane receptors for FSH (Orth & Christensen, 1977) while cytoplasmic and nuclear receptors for androgens have been demonstrated in isolated Sertoli cells (Sanborn et al, 1977; Tsai et al, 1977).

8.4 Mechanism of action of follicle stimulating hormone

The application of cell culture techniques and methods for preparing Sertoli cell preparations (Welsh & Wiebe, 1975; Steinberger A. et al, 1975) has facilitated the studies of molecular mechanisms of FSH action on the seminiferous tubules. The events following specific FSH receptor binding in Sertoli cell membranes are similar to that in LH binding with Leydig cells with activation of adenyl cyclase and cyclic AMP dependent protein kinase (Means et al, 1976). This series of biochemical events results in increased RNA and protein synthesis in Sertoli

cells which include a number of specific proteins. Androgen binding protein (ABP), an androphilic macromolecule with physicochemical properties characteristic of a transport protein (Hansson et al, 1975; Sanborn et al, 1975) and plasminogen activator (Lacroix et al, 1977) are secreted into the rete testis fluid by the Sertoli cells. A further two specific proteins - a protein kinase inhibitor and γ glutamyl transpeptidase, however, remain within the Sertoli cells (Beale et al, 1977; Hodgen & Sherins, 1973). FSH also regulates the cytoskeletal component of Sertoli cells via stimulation of a calcium-dependent regulator protein (calmodulin) coupled to the adenyl cyclase-phosphodiesterase system. By reorganization of the microtubules and microfilaments of the Sertoli cell cytoskeletal FSH may influence the cell shape, motility and exocytosis (Means et al, 1978 & 1980).

All the effects of FSH discussed above have been demonstrated only in Sertoli cells from immature rat, and little if any FSH action could be elicited by twenty days of age, the time at which the Sertoli cell tight junctions are formed (Means et al, 1980). This is also the time when germ cell meiosis is first encountered, marking the onset of full spermatogenesis. The age-dependent change in Sertoli cell FSH responsiveness may be due to alterations in the isoforms of cyclic AMP-phosphodiesterase (Means et al, 1980) but the apparent inactivity of adult Sertoli cells in vitro is one of the most important riddles to solve in our understanding of testicular function.

8.5 Mechanisms of hormonal action on spermatogenesis

From the physiological studies defining the hormonal requirements for normal spermatogenesis and the present understanding of molecular mechanisms of action of these hormones in the seminiferous tubule, it may be possible to formulate some tentative model for the hormonal control of spermatogenesis. A great deal of attention has been focussed on the androgen binding protein synthesis and secretion by Sertoli cells since this is stimulated not only by FSH but also testosterone (Elkington et al, 1975; Means et al, 1976; Louis & Fritz, 1977). Furthermore, the post-hypophysectomy reduction in testicular ABP concentration can be prevented if androgen replacement is started immediately after the operation. Otherwise if testicular regression has occurred, restoration of ABP production requires both FSH and androgens (Elkington et al, 1975; Weddington et al, 1975). Thus the Sertoli cell requirement for ABP production closely parallels the hormonal requirement for spermatogenesis in rats. Taken together, these observations suggest that ABP may play a central role in spermatogenesis although the exact nature of its function in the seminiferous tubule is unclear. Androgen binding protein, with its high affinity for testosterone, may prevent the rapid metabolism of androgen to oestradiol or 5α-reduced metabolites so that a high intratesticular concentration of testosterone can be ensured. There are obvious deficiencies in this highly speculative model of seminiferous tubule function. The action of testosterone is unlikely to be directly on the

germ cells (Lyon et al, 1975) and the mechanism of action of androgens on spermatogenesis is completely unknown. Even the fundamental tenet that high intratesticular testosterone concentration is required for normal spermatogenesis has recently been challenged (Cunningham & Huckins, 1979). In man and other primates, a specific testicular or epididymal ABP distinct from SHBG has been difficult to identify (Vigersky et al, 1976). Clearly, much more basic information remains to be learned before the mechanism(s) of hormonal control of spermatogenesis can be fully appreciated.

CHAPTER 9

GONADOTROPHINS

In vertebrates, the pituitary synthesizes two gonadotrophins - luteinizing hormone (LH) and follicle stimulating hormone (FSH) which regulate gonadal functions.

9.1 Isolation and purification

Early attempts at isolation of the glycoprotein gonadotrophins using ammonium sulphate fractionation or precipitation by buffered ethanol produced impure preparations which were inadequate for structural and functional analysis. The introduction of more sophisticated peptide chemical methodology such as ion exchange and molecular exclusion, starch gel and polyacrylamidegel electrophoresis and isoelectric focusing yielded reasonably pure preparations of gonadotrophins for chemical and biological analyses followed by the development of radioimmunoassays. Demands on gonadotrophins for clinical purposes prompted investigators to seek alternative sources of these hormones due to the meagre supplies of animal and human pituitaries. Urine from post-menopausal women proved the most rewarding although these preparations obtained (Human Menopausal Gonadotrophins - HMG) contain both LH and FSH which are not easily separated from each other. Nevertheless, preparations of HMG are suitable for clinical use as well as acting as reference standards for bio and immunoassays.

Other non-pituitary sources of gonadotrophic hormones include human chorionic gonadotrophin (hCG) from the urine of pregnant women, with its LH-like activity (Diczfalusy & Troen, 1961). The relative abundance of HCG in easily available pregnancy urine and its similarity in biological actions to pituitary LH make it the most widely used glycoprotein hormone for the study of structure-function relationships and mode of action of this class of hormone.

9.2 Physico-chemical properties of gonadotrophins

Gonadotrophins, either crude or highly purified, contain more than one immunological and biologically active moieties which are regarded as different forms or molecular species of the same hormone. This may reflect the presence of different forms of gonadotrophins stored in the pituitary or result from artefacts during extraction and purification. Whatever the cause, hormonal heterogeneity of gonadotrophins, as indeed in all peptide hormones, is a phenomenon that endocrinologists have to contend with constantly in their enquiries.

For the same reasons, there is no general agreement as to the molecular weight of gonadotrophins which was estimated to be between 32,600 to 52,480 for FSH and 26,000 to 41,690 for LH (Wide, 1976). The carbohydrate content is 15% in LH and 30% in FSH; these are covalently linked to the peptide chains. The important observation of Li and Starman (1964) that ovine LH readily dissociates in acid pH paved the way for the subsequent discovery that

all glycoprotein hormones including LH and FSH reversibly dissociate into dissimilar α and β subunits each of 100 amino acids when treated by acidic or high molarity reagents that do not disrupt the covalent bonds. Dissociated α and β subunits have little biological activity which is restored when they are re-associated under appropriate conditions. The nature of this restored activity is dependent on the β subunit (Liao & Pierce, 1970; Saxena & Rathnam, 1971) since the α subunits in all human glycoprotein hormones have identical amino acid sequence (Shome & Parlow, 1974). This within-species structural similarity of the α subunits accounts for the cross-reactions commonly encountered with antibodies raised against any one of the glycoprotein hormones with the other three (Vaitukaitis et al, 1972). On the other hand, β subunits of human glycoprotein hormones show antigenic similarities with those of the corresponding hormones of other species (Vaitukaitis et al, 1972). Thus within-species immunological cross-reactivity resides in the α subunit and the between-species immunological cross-reactivity resides in the β subunit. This phenomenon is the basis for enhancing the specificity in radioimmunoassays of gonadotrophins using heterologous systems, e.g. labelled ovine FSH and anti-human FSH antiserum in measuring human FSH (L'Hermite & Midgeley, 1971).

Steroid hormones circulate in plasma largely as complexes with specific and non-specific carrier proteins. This may be related to their insolubility in water even though their physiological concentrations do not normally

exceed their aqueous solubility. The lipid solubility of steroids is compatible with their mainly intracellular sites of action requiring easy penetration of the target cell membrane. Peptide or glycoprotein hormones, in contrast, are 1000 to 100 million times more soluble in plasma than steroids and it is generally accepted that the native hormones are not bound to any circulating carrier proteins (Berson & Yalow, 1970). Binding of some moiety of radio-labelled hormone to α_2 macroglobulins may reflect hormonal damage by plasma peptidases. The much higher MCRs of gonadotrophins compared to steroids are compatible with the above arguments.

Secretion and metabolic clearance rate methods have been extensively applied to the study of steroid hormones where the native and labelled molecules are well characterized by chemical and chromatographic purification procedures. The application of these techniques to peptide hormones are fraught with practical pitfalls, due to the heterogeneity of gonadotrophins in the circulation and the unavoidable presence of damaged components from radiolabelling whose plasma half-lives are spuriously prolonged because of their tendency to protein binding. Furthermore, for peptide hormones in the physiological and pharmacological ranges, metabolic clearance rates seldom fluctuate and very few agents or factors significantly influence their metabolic degradation (Berson et al, 1956). Coupled with the rapid metabolic turnover of peptide hormones ($T\frac{1}{2}$ usually in minutes), any rise and fall in plasma concentrations can effectively be

regarded as indicative of increase or decrease in rates of glandular secretion. Using constant infusions of highly purified labelled and native gonadotrophins, the MCRs of gonadotrophins in normal adult males were estimated to be 64.2 L/day for LH and 9-14 L/day for FSH. The corresponding secretion rates were 85 (range 45-123) I.U. and 25 (6-66) I.U. of 2nd IRP HMG respectively (Burger et al, 1974). The proportion of gonadotrophin production excreted in urine was around 40%. Both intact hormone and degraded products as well as subunits with immunological activity are excreted. Although measurements of gonadotrophin in the urine generally reflect the plasma concentrations reasonably accurately, the renal excretion rates are strongly influenced by changes in renal function (Wide & Thoren, 1972). In addition, the normally large variation in pH and solute concentrations in urine may interfere with the immunoassay specificity.

CHAPTER 10

NEUROENDOCRINE CONTROL OF GONADOTROPHIN SECRETION

The posterior pituitary and the adrenal medulla, of all endocrine glands, are the only ones which receive a rich nerve supply influencing their secretory activity. Yet the functions of the gonads are critically influenced by environmental factors such as light, temperature and the presence of mates (Marshall, 1936, 1942). The attachment of the pituitary gland to the hypothalamic area of the brain provides a key anatomical link whereby sensory stimuli from the external environment can be relayed through the CNS neural pathways to the gonads. This integration of neural inputs into an endocrine functional response is the core of the neuroendocrine concept of control of reproduction.

10.1 Neurovascular control of anterior pituitary function

Repeated attempts between 1935-1955 to define a nerve supply linking the hypothalamus and pituitary led to the inevitable conclusion that it did not exist. Attention was consequently turned to humoral or chemical transmission between the two sites, prompted by Sawyer's experiments (Sawyer et al, 1947) showing that ovulation in rats was prevented by α adrenergic blocking agents and stimulated by intraventricular injection of noradrenaline. A delicate portal vessel system between the primary capillary plexus in the median eminence and the sinusoids of the anterior pituitary was first described by Popa & Fielding (1930, 1933). This was subsequently confirmed

by Wislocki and King (1936). Later, it was demonstrated that the hypophysial portal vessels were widely distributed amongst vertebrates (Green, 1951) and that they carried blood from the median eminence to the pituitary (Green & Harris, 1949). Thus the gross anatomical basis for the neurovascular control of the anterior pituitary was established. This was further supported by ultrastructural studies demonstrating an abundance of nerve endings containing secretory vesicles in close contact with endothelium of the primary capillaries with fenestrations typical of absorptive and secretory structures (Barry & Cotte, 1961).

The essential role of this hypophysial portal supply to normal anterior pituitary function was established by the elegant work of Harris and Jacobson (1952). They found that anterior pituitary tissue transplanted to ectopic sites in the body failed to function. However if the same tissue was transplanted under the median eminence where revascularization by the portal circulation can occur, normal function was restored. This series of experiments prompted the full acceptance of the neurovascular theory and set the stage for the search for hypothalamic compounds transported in the portal vessels which stimulate pituitary hormone production.

10.2 Isolation, purification and synthesis of Gonadotrophin Releasing Hormone (GnRH)

Drawing an analogy with the posterior pituitary's synthesis of neurosecretory peptide hormones, it was not

long before the successful isolation of peptide-containing hypothalamic extracts capable of evoking ovulation and pituitary LH secretion was achieved (McCann et al, 1960; Campbell et al, 1961). Despite the minute quantities of GnRH present in the hypothalamus, adequate amounts of the extract were obtained to allow the determination of the structure of porcine GnRH in Schally's laboratory (Matsuo et al, 1971a). The linear decapeptide structure was confirmed shortly by Guillemin's group in ovine hypothalamus extracts (Burgus et al, 1972). The synthesis of the peptide sequence was quickly accomplished (Matsuo et al, 1971b; Geiger et al, 1971) and the potent action in stimulating not only LH but also FSH has been confirmed in all vertebrate species examined with a seemingly complete lack of species specificity in action and structure (Fraser, 1981). The availability of synthetic GnRH for experimental and clinical use was one of the most significant scientific achievements in the last decade for which Schally and Guillemin were jointly awarded the Nobel prize.

Under most physiological situations, the LH response to GnRH is much greater than that of FSH. However, in some instances, FSH secretion can be dissociated from LH with preferential release or suppression of the former (Chappel & Barraclough, 1976 & 1977). Although these could be taken as evidence supporting the existence of a separate FSH-RH, the more likely explanation is the differential effects of steroid or other substances on the pituitary response to a single GnRH. The extensive

biochemical analyses of Schally's group has led him to conclude that GnRH represents the bulk of FSH-RH activity in porcine and bovine hypothalami (Schally et al, 1976).

10.3 Location of GnRH

Topographic studies of the brain have all shown that the highest concentration of GnRH occurred in the axons of the lateral palisade zone of the median eminence where the nerve endings contain large diameter (100-300 nm) GnRH secretory granules similar to synaptosomes (Arimura, 1977; Brownstein, 1977; Krulich & Fawcett, 1977). In rodents, the principal area bearing GnRH-containing neurones is the preoptic-anterior hypothalamic area from where axons run in the hypothalamo-infundibular tract to end on proximal capillaries of the portal system. The other important area rich in GnRH producing neurones in primates is the arcuate nucleus - medial basal hypothalamus region. Axons from this area also project into the median eminence. The presence of GnRH in nerve fibres outside the hypothalamus, e.g. Amygdala and pineal, and in non-neural reproductive tissue has led to intensive studies of possible functions of GnRH outwith the gonadotropes of the pituitary (for review see Hsueh & Jones, 1981).

10.4 Biosynthesis and release of GnRH

Although the in vitro synthesis of GnRH by rat hypothalamic fractions has been demonstrated (Johansson et al, 1973), the subcellular site, molecular mechanism and

regulatory processes of GnRH synthesis have not been studied in detail. However, the relevant mechanisms are likely to be similar to other hypothalamic peptide hormones (e.g. vasopressin) with ribosomal synthesis of a larger prohormone which is cleaved by peptidases before being packaged for secretion in the Golgi apparatus.

The release of GnRH is the end result of the complex interactions between environmental, psychological and steroidal as well as non-steroidal gonadal feedback signals with the neurosecretory neurones in the hypothalamus. The decapeptide synthesized in the cell bodies passes down the axons by axoplasmic flow to the nerve endings at the median eminence. There the hormone is concentrated in large secretory granules prior to release into capillaries of the portal vessels. This release is presumably evoked by an action potential originating in the cell body in response to stimuli from the afferent system. Hence the process of discharge of secretory granules from nerve-endings probably follows the all-or-none phenomenon for nerve impulse conduction and the amount of hormone released into the portal circulation will be dependent on the frequency of neuronal pulsatile discharge (Hayward, 1977).

The extremely low concentrations of GnRH in the systemic circulation render its measurement in peripheral blood unreliable. However with the techniques of pituitary stalk blood sampling perfected by Fink in Oxford and Porter in Dallas, several studies have been able to demonstrate the tonic pulsatile

nature of GnRH secretion and the cyclic preovulatory GnRH surge in the hypophysial portal circulation (Carmel et al, 1976; Sarkar et al, 1976; Eskay et al, 1977; Neil et al, 1977; Tsou et al, 1977), mirroring the two well-described patterns of LH secretion in the systemic circulation in the female reproductive cycle. These important observations confirmed the physiological role of GnRH secretion and also validated the use of pulse frequency of pituitary LH secretion as an index of hypothalamic activity.

10.5 Neurotransmitter control of GnRH secretion

Although it was the hypothalamic peptide hormone that captured the attention of most investigators in the last decade, it should be emphasized that the original experiments which fostered the concept of neurohumoral transmission between the brain and pituitary suggested that adrenergic or cholinergic neurotransmitters were the mediators of this communication (Sawyer et al, 1949; Sawyer, 1952; Markee et al, 1952). Subsequently, many studies have shown that a wide variety of centrally-acting agents can block ovulation as well as pulsatile GnRH secretion (Bhattacharya et al, 1972; Drouva & Gallo, 1976; Arendash & Gallo, 1978) confirming that CNS neurotransmitters are important in the control of GnRH secretion from the hypothalamus and thus reproduction.

Noradrenaline containing fibres run from the lateral tegmental area of the brain stem to the GnRH-containing areas of the hypothalamus (Fuxe & Hökfelt, 1969).

Extensive experimental evidence in the rat in eliciting and blocking the preovulatory LH surge has confirmed an excitatory role of noradrenaline on the GnRH-secreting neurone (McCann & Ojeda, 1976).

The highest concentration of dopamine in the brain is located in the median eminence where nerve fibres of the tuberoinfundibular tract with neurones in the arcuate and periventricular areas terminate (Fuxe & Höckfelt, 1969). The proximity of dopamine and GnRH-containing nerve fibres in the median eminence raised the possibility that axon-axon synapses may regulate the neurosecretion of GnRH. The early in vivo and in vitro experiments of McCann suggested that dopamine stimulated GnRH from the hypothalamus of rats (McCann & Ojeda, 1976). These results however could not be confirmed by others who found a consistent inhibitory effect of dopamine in several species (Fuxe et al, 1976; Fuxe et al, 1978). More recent studies by McCann and colleagues showed that dopamine may have different actions on GnRH neurones depending on steroid environment, dosage of dopamine, the individual agonist or antagonist used and the route of administration (Vijayan & McCann, 1978a & b). Dopamine is also secreted directly into the hypophysial portal circulation (Shaar & Clemens, 1974) where it inhibits the pituitary response to GnRH (Yen et al, 1977; Judd et al, 1978). This may further confound any direct excitatory effect of dopamine on GnRH production.

Systemic and intraventricular injection and the use of specific antagonists have shown that serotonin may have

an inhibitory effect on GnRH secretion (McCann & Ojeda, 1976). Electrical stimulation of the arcuate nucleus induced pulsatile release of LH which is suppressed by serotonin (Gallo & Moberg, 1977). Oestrogens alter serotonin turnover, increasing it at night and reducing it during the day (Fuxe et al, 1976). This may be important in the LH sleep/wake secretory rhythm. However most of these studies were performed with pharmacological doses and the physiological role of serotonin and other putative neurotransmitters in the control of GnRH secretion remains to be proven.

One of the most intriguing issues in neuroendocrinology is posed by the discovery of a wide variety of hypothalamic, pituitary, gastrointestinal and opioid peptides in the brain making them potential candidates as neurotransmitters and neuromodulators (McCann, 1980; Kreiger, 1980). The site of origin, mechanism of biosynthesis and physiological roles of these CNS peptides are just beginning to be studied and it is too early to say whether any of them would play a significant role in the physiological control of GnRH or gonadotrophin function.

Another class of compound, the prostaglandins, is found in high concentrations in the median eminence (Ojeda et al, 1979). Although PGE_2 and E_1 can stimulate LH release directly from the pituitary (Labrie et al, 1976), strong evidence suggests that their principal site of action is the induction of GnRH secretion (McCann et al, 1976). It has been postulated that activation of

noradrenaline release stimulates post-synaptic production of PGEs which in turn enhances the release of GnRH directly or via cyclic AMP (Ojeda & McCann, 1978).

10.6 Mechanism of action of GnRH

As in other peptide hormones, GnRH binds to specific cell surface receptors (Grant et al, 1973). However, the predominant low affinity ligand binding and tracer degradation prevented the accurate measurement and meaningful studies of the physiologically important high affinity binding sites on the gonadotrope cell membranes. In the last few years, the use of radioiodinated agonist analogue of native GnRH as the ligand in radioreceptor assays has eliminated these methodological obstacles (Clayton et al, 1979) and the physico-chemical characteristics of the physiological GnRH receptor defined. Using this approach, evidence supporting the existence of mechanisms regulating GnRH receptor numbers in the pituitary by gonadal steroids and endogenous GnRH secretion has been obtained (for review see Clayton & Catt, 1981). The post-receptor mechanisms of GnRH action is at present unclear. Data on the role of cyclic AMP and cyclic GMP as the intracellular second messenger after membrane receptor binding are conflicting (Wakabayashi et al, 1973; Conn et al, 1979; Noar et al, 1978; Noar & Catt, 1980). Recent evidence reviewed by Conn et al (1981) suggests that although cyclic nucleotides may be involved in the regulation of GnRH-induced LH biosynthesis, they could be readily uncoupled from LH

release. There is growing support at present for the role of cytosolic calcium and its intracellular binding protein, calmodulin, being the mediator of GnRH-induced gonadotrophin release in the pituitary gonadotropes (Marian & Conn, 1979; Conn et al, 1979). Calmodulin is known to regulate the assembly and disassembly of microtubules (Means & Dedman, 1980) which are important in the secretion of gonadotrophins (Khar et al, 1979). Although the pituitary gonadotrophin store is rapidly depleted implying that GnRH also induces de novo hormone synthesis (Labrie et al, 1973), nonetheless short term treatment of pituitary with puromycin or cycloheximide did not decrease the initial or acute gonadotrophin release in response to GnRH (Vale et al, 1968). This is the basis for the concept of the existence of two pools of secreted LH - the initial release of hormone from a readily releasable pool followed by the secretion of stored or newly synthesized hormone from a second pool after prolonged GnRH stimulation (Bremner & Paulsen, 1974; Wollensen et al, 1976; Yen, 1977). These two functional rather than anatomical compartments of LH are closely integrated as illustrated by the self-priming phenomenon where successive GnRH stimulation shifts the storage into the releasable pool and increases the secretory response to subsequent stimulation (Aiyer et al, 1974; Fink, 1979).

10. 7 Metabolism of GnRH

The plasma clearance of exogenous radiolabelled GnRH is extremely rapid. At least two components can be

recognized with half life of the initial fast component of four minutes and a second slower component of fifty minutes (Nett et al, 1973; Keye et al, 1973). This pattern is similar in all species studied (Arimura et al, 1974). The highest tissue uptake of circulating GnRH occurs in the liver and kidney indicating that these organs may be the primary sites for GnRH inactivation (Yukitaka et al, 1973).

CHAPTER 11

BIORHYTHM OF REPRODUCTIVE HORMONE SECRETION

The classical studies and thinking based on bioassay of urinary hormones fostered the concept that hormones are secreted by endocrine glands at a steady constant rate throughout the twenty-four hours. This is now known to be untrue. Indeed, rhythmic fluctuations in hormone synthesis and secretion are characteristic of mammalian endocrine systems and may be part of some fine-tuning control mechanisms at the target cell or receptor level. Despite this upsurge in interest in the rhythmic pattern of hormone secretion, it should be said that the valuable data from experiments based on steady state steroid dynamics remain valid since the estimations on daily blood production rates and conversion constants can allow for or integrate the fluctuating levels of hormones in the circulation. Hormones which regulate reproductive functions have several recognizable patterns of variation with different periodicities superimposed on each other.

11.1 Circhoral rhythm

With the availability of simple, sensitive and precise radio-ligand assays on small volumes of biological fluids, repeated determinations of circulating hormone concentrations at frequent intervals became possible. Using the technique of frequent blood sampling (Vankirk & Sassin, 1969) the rhythmic or pulsatile oscillation of plasma LH concentration was first

demonstrated in ovariectomized Rhesus monkeys (Dierschke et al, 1970). These workers used the term circhoral rhythm to describe the hourly LH oscillations in peripheral plasma and this has now been expanded to include other high frequency hormone rhythms even though the periodicity usually exceeds one hour. The existence of rhythmic fluctuation of LH concentration was soon confirmed in gonadectomized and intact animals in other species (man: Nankin & Troen, 1971, Midgley & Jaffe, 1971; bull: Katongole et al, 1971; rat: Gay & Sheth, 1972; ram: Sandford et al, 1974; rabbit: Moore & Younglai, 1975). In the adult human male, data from many different studies are essentially in agreement (for review see Baker et al, 1975 and Judd, 1979). A typical LH pulse rises sharply to a peak in five to forty minutes with increments of 30-300% followed by a slower decline over one half to four hours before reaching basal levels. The apparent decay time of 100 to 130 minutes for LH pulses exceeds the half-life (29-63 minutes) of the exogenous radiolabelled hormone in hypophysectomized subjects (Kohler et al, 1968; Schalch et al, 1968; Yen et al, 1968), suggesting that pulsatile secretion may occur on a background of modest continuous secretion (Yen et al, 1972a; Vande Wiele & Ferin, 1974). The interval between LH pulses varied between one to six hours with an average of 100 minutes (Santen & Bardin, 1973; Baker et al, 1975).

In the intact subject, FSH pulsatile secretion is less marked with a slower rise and non-linear slopes of decay (Yen et al, 1972b). This pattern is compatible with

(1) lower absolute levels of FSH; (2) more moderate response of FSH to GnRH; (3) longer plasma half-life of FSH (Yen et al, 1970) and (4) different modulating effects of gonadal steroids at the pituitary. Despite these features, LH and FSH are usually closely correlated temporally (Yen et al, 1972b; Santen & Bardin, 1973; Alford et al, 1973) reinforcing the belief that a single releasing hormone stimulates the synthesis and release of both gonadotrophins (Schally et al, 1976).

The secretion of testosterone is also pulsatile to a certain extent in man although the pattern is less well-defined than that of LH being of smaller magnitude and more irregular in frequency (for review see Baker et al, 1975 and Judd, 1979). By increasing the frequency of sampling to two minute intervals, Smith et al (1974) were able to show distinct and rapid oscillations in testosterone which was not apparent with lower sampling frequencies. Although the biological significance of this finding is obscure, it serves to emphasize the importance of the optimal sampling frequency for the study of pulsatile secretion depending on the pulse interval and the plasma half-life (Goldzieher et al, 1976).

Variations in plasma concentrations of hormones may be due to changes in production/secretion rate, metabolic clearance rate or volume of distribution. Since the MCR of gonadotrophins (Kohler et al, 1968; Coble et al, 1969) and testosterone (Southren et al, 1968) remain relatively constant at most times and the effect of posture and

changes in plasma volume could not possibly account for the minute to minute changes in hormone concentration, the circhoral or ultradian variation of these hormones must therefore reflect changes in secretion rate. Although direct proof by measuring changes of hormone concentration with time in the venous effluent of the relevant glands is still lacking, it is now generally accepted that hormones showing episodic changes in plasma concentrations are secreted in a pulsatile fashion.

Episodic secretion of gonadotrophins may be an intrinsic property of the pituitary or the result of pulsatile stimulation by hypothalamic GnRH or both. The former mechanism is supported by the persistence of short term fluctuation of LH during continuous GnRH infusion (Mortimer et al, 1974; Vande Wiele & Ferin, 1974; Bremner et al, 1976). The evidence for pulsatile GnRH stimulation however is much more convincing. Pituitary stalk section without revascularization leads to rapid falls in gonadotrophin levels (Vaughan et al, 1980). More selective inhibition of GnRH by active or passive immunization with anti-GnRH antisera abolishes pulsatile LH secretion in all species studied (reviewed by Fraser, 1981). Although these experiments demonstrated the obligatory role of hypothalamic GnRH in pituitary gonadotrophin synthesis and secretion, it remains to be proven whether GnRH also stimulates pulsatile gonadotrophin release. It has already been mentioned in the last chapter that measurement of GnRH in blood collected from the hypophysial portal circulation in monkeys showed that this neuro-

hormone is indeed released in a pulsatile manner with pulse intervals between one and three hours (Carmel et al, 1976). A separate study using a different technique of portal blood sampling which does not require pituitary stalk section was able to demonstrate a temporal correlation between portal blood GnRH and peripheral blood LH during the proestrous LH surge in rats (Sarkar et al, 1976).

Unlike the bull or the ram (Katongole et al, 1971; Sandford et al, 1974), a close temporal relationship between episodic LH and testosterone secretion in adult men is not immediately apparent (Alford et al, 1973; Murray & Corker, 1973; Naftolin et al, 1973; de Lacerda et al, 1973). This is somewhat surprising in view of the fact that LH is the major stimulus for testicular steroidogenesis. One possible explanation may be a rather sluggish Leydig cell response in humans to LH. This is suggested by the significant correlation between LH and corresponding testosterone concentrations one to four hours later (Rowe et al, 1975). This inertia in testicular response is further supported by the poor testosterone response to increased LH produced by GnRH (de Kretser et al, 1975a) and LH infusion (Marshall et al, 1973). The exact reasons for the delayed or attenuated testicular response to LH in humans is not clear.

The biological significance of these high frequency oscillations in hormones of the hypothalamic-pituitary-testicular axis has recently been elegantly demonstrated. In rhesus monkeys with destructive lesions in the arcuate

nucleus, pulsatile exogenous GnRH stimulation resulted in sustained increases in LH and FSH whereas continuous administration led rapidly to a fall off of pituitary gonadotrophin response (Belchetz et al, 1978). Similar studies in patients with hypogonadotrophic hypogonadism also confirmed the importance of intermittent or pulsatile GnRH administration in optimizing the treatment regime (McArthur & Crowley, 1980). Presumably, the intermittent stimulation of GnRH minimizes the degree of receptor down regulation (Catt & Dufau, 1976) and allows the self-priming and trophic effects of GnRH on the gonadotropes to develop. This argument also applies to the LH stimulation of Leydig cells (Catt et al, 1980) but as yet the importance of these negative regulatory effects in the physiological control of the testis is unknown. From the satisfactory virilization of hypogonadal patients by exogenous androgens, it seems unlikely that pulsatile stimulation of androgen target organs is essential to their normal function. It may be said that the further is the hormonal signal from the brain, the less important is the temporal or frequency modulation component of the stimulus. This is compatible with the view that the normal functioning of the HPT axis involves the transformation of an electrochemical signal from the brain to the steroid molecule decoding of genetically determined functions of various target organs.

11.2 Circadian rhythm

Early studies with sampling frequencies of four to

six hours suggested the presence of a circadian periodicity in plasma LH and FSH concentration (Faiman & Ryan, 1967; Faiman & Winter, 1971; Saxena et al, 1968). These reports however could not be confirmed by more frequent sampling (20 minute intervals) in adult males (Krieger et al, 1972; Boyar et al, 1972a; Alford et al, 1973). In young men, the magnitude of LH pulses seems to be highest just before waking in a twelve hour overnight study (Nankin & Troen, 1972). This pattern is even more pronounced in pubertal boys in whom Boyar et al (1972b) showed that augmented LH secretion clearly occurred throughout the period of sleep. This highlights the importance of neuroendocrine factors in the control of gonadotrophin secretion and may have important theoretical and clinical implications.

Although early reports disagreed due to inadequate sampling frequencies, there is now little doubt that a circadian rhythm in plasma testosterone concentration exists with peak levels at 03.00 - 10.00 h and trough at 16.00 - 02.00 h (Nieschlag, 1974; Licoln et al, 1974). Compared to that of circhoral rhythm, the amplitude of the circadian rhythm is small - 20-40%. Furthermore, the timing of the testosterone peak and trough shows considerable variation within and between patients.

The mechanism of the circadian variation in plasma testosterone does not depend on posture or activity-related changes in MCR (Lipsett et al, 1966; Southren, 1967 & 1968) or adrenal steroidogenesis (Judd et al, 1974). Since 95% of testosterone blood production is

secreted by the testes (see Chapter 4), the circadian rhythm of testosterone results from changes in the rate of testosterone secretion. The absence of similar rhythmic changes in gonadotrophins and the different patterns of prolactin secretion (see Chapter 13) tend to exclude any direct effects of these hormones in the daily cycle of testosterone secretion. The effect of catecholamines on testicular blood flow may contribute to the circadian variation in testosterone secretion since a similar cycle in urinary excretion of catecholamines has been demonstrated (von Euler et al, 1955). Seventeen-α-hydroxyprogesterone levels fluctuated in parallel with testosterone whereas androstenedione and DHEA, being adrenal androgens, were more closely associated with the cortisol periodicity (Sjöberg et al, 1979).

11.3 Circannual rhythm

Many animal species have seasonal rhythms in their reproductive process in order that rearing of offspring is timed to coincide with optimal environmental conditions in terms of climate, food supply and predation pressure. These environmental variables are unreliable as signals for animals to determine the season. Hence a wide variety of animals have evolved a system relying on the relatively stable annual cycle in daylength to synchronize their seasonal reproductive cycles. Hence changes in photoperiod can induce regression and development of the gonads via its effect on hypothalamic-pituitary activity. The mechanism underlying this

neuroendocrine phenomenon is unclear. It requires a photoreceoptor organ - retina in mammals, an endogenous circadian clock involved in the measurement of day-length and a mechanism of information transfer from the endogenous clock to the hypothalamic-pituitary unit. There is increasing evidence that the pineal gland and its indolamine or peptide secretory products may be intimately involved in the latter function and mediate the antigonadotrophic action at the appropriate seasons (Licoln & Short, 1980; Turek & Campbell, 1979; Reiter, 1980).

In humans, reproduction is a continuous process after puberty. Although there is a gradual decline in reproductive function with age, the marked seasonal changes seen in some animals are absent. However, the activation of gonadotrophic functions during the dark phase of the twenty-four hour cycle in human puberty has certain features akin to the seasonal activation of gonadal function in animals. There is some evidence that a circannual rhythm in testicular function may exist in humans. Smals et al (1976) studied fifteen adult men at three monthly intervals and found significantly higher results in July-October than January-April. The magnitude of this variation is small (16-22%). Reinberg et al (1975) found similar results in five men. Burger et al (1972) observed higher testosterone levels in the Australian spring rather than winter. Bearing in mind that these studies were based on single samples of testosterone estimated at various intervals (three times

weekly to three monthly) and that the amplitude of the variation is small, it is doubtful whether a true circannual rhythm of testicular function exists in the human male.

CHAPTER 12

FEEDBACK CONTROL OF GONADOTROPHIN SECRETION

The early studies of Moore & Price (1932) and Greep & Jones (1950) on the effect of gonadal hormones established the concept of feedback control in endocrine physiology which has since been applied to virtually all hormone producing systems. Thus removal of the adult gonads leads to increased gonadotrophin secretion while the administration of exogenous gonadal steroids in sufficient quantities will suppress gonadotrophin secretion in intact or gonadectomized animals (for review see Davidson, 1969; Brown-Grant, 1977). It was suggested that the negative feedback system operates physiologically to maintain a constant level of gonadotrophin and steroid hormone in the circulation. Although the principle of negative or inhibitory feedback is firmly accepted, there are many important details in the mechanism still to be clarified. The quantitative study of the dynamics of negative feedback of gonadotrophin secretion only became possible in the last decade with the availability of sensitive radioimmunoassays. The discovery of pulsatile gonadotrophin secretion added a new dimension to the analysis of feedback mechanisms while the interconversion of secreted gonadal steroids further complicated the picture. The response of LH and FSH under different physiological or pathological conditions may be discordant. The molecular mechanisms and sites of gonadal steroid feedback at the brain, hypothalamus and pituitary and the

existence of short-loop feedback by gonadotrophins and ultrashort-loop feedback by GnRH are areas that are just beginning to be explored. Thus what appears at first glance to be a simple on-off mechanism of steady-state secretion is in reality a highly complicated one with multiple integrated levels of function and control.

12.1 Feedback control of LH

12.1.1 Negative feedback

Castration in adult men leads to a prompt fall in plasma testosterone and although both LH and FSH increase within 24 to 72 hours, peak levels are reached only in the subsequent three to four weeks (Burger et al, 1972; Walsh et al, 1973). Changes in the pulsatile pattern of gonadotrophin secretion may be expected in castrated or agonadal men but, surprisingly, no report in the literature has examined this issue in any detail. In animals, although pulsatile gonadotrophin secretion has been well described in a number of species, there is only one study which compared the frequency and amplitude of pulsatile gonadotrophin secretion in castrated and intact rams - both parameters were increased (Licoln & Short, 1980). In the human postmenopausal or agonadal female, the frequency of LH pulses was similar to that in the follicular phase of premenopausal women but the amplitude of each pulse was increased (Yen et al, 1972b; Santen & Bardin, 1973).

Since it is the major secretory product of the testis, testosterone has come to be regarded as the

principal negative feedback signal in the control of pituitary LH secretion. However, it has recently been demonstrated that both oestradiol and dihydrotestosterone, the two steroids derived from metabolism of testosterone at target organs, have distinct and independent negative feedback effects on gonadotrophin secretion (Santen, 1977; Santen & Ruby, 1979; Winters et al, 1979). The relative importance of the three steroids in the physiological feedback control of gonadotrophin secretion is at present unknown but these studies emphasized the point that androgens and oestrogens exert independent effects on gonadotrophin secretion probably via different mechanisms and perhaps at different sites. So far, it has been difficult to differentiate between a pituitary or hypothalamic site of action of steroid negative feedback. The suppressive effect of testosterone on the pituitary response to exogenous GnRH stimulation in man and animals (Caminos-Torres et al, 1977; Debeljuk et al, 1972) and the reduction in LH pulse frequency after testosterone infusion in man (Santen, 1977) suggest that both the pituitary and hypothalamus can mediate the negative gonadal steroid feedback independently. Steroid receptors are detectable in the pituitary, hypothalamus as well as the brain (McEwen, 1980). It is possible that steroids may modify gonadotrophin secretion via modification of electrical activities of CNS neurones, neurotransmitter concentrations and turnover or alteration in GnRH receptor populations in the pituitary. These novel aspects of steroid action in relation to gonado-

trophin and GnRH control are little understood at present. The multiple sites and possible mechanisms of action of steroid negative feedback amply illustrate the complexity of gonadotrophin regulation, which ultimately represents the end result of interactions between neuro-endocrine and gonadal influences on the hypothalamus and pituitary.

12.1.2 Positive feedback

The cyclical nature of gonadotrophin secretion in the female is characterized by a positive feedback mechanism in which oestradiol triggers the preovulatory LH release. This phenomenon is absent in intact male rat and ram (Caligaris et al, 1972; Karsh & Foster, 1975) but could be elicited by supraphysiological doses of exogenous oestrogen in monkeys and man (Stearns et al, 1973; Karsh et al, 1973; Hodges, 1980; Barbarino et al, 1979, 1980). In the subprimate species at least, sexual dimorphism is descernable in the steroid-gonadotrophin interaction and the function of the hypothalamic-pituitary unit. Studies in the female rat and ewes showed that prenatal or neonatal exposure to androgens (or oestrogens) abolished the positive feedback mechanism in adult life and these females secrete gonadotrophins acyclically (Gorski, 1971; Short, 1974). This organizational effect of gonadal steroids on the brain and hypothalamus is akin to that described for the differentiation of sexual behaviour. In female rhesus monkeys and humans, pre or postnatal exposure to androgens failed to abolish positive feedback in adult

life (Treloar et al, 1972; Goy & Resko, 1972; Erhardt et al, 1977). These studies illustrate that gonadal steroids can exhibit both inhibitory and stimulatory effects on the hypothalamic-pituitary unit and may also direct the subsequent pattern of gonadotrophin secretion by virtue of its action on the developing brain.

12.2 Inhibin

FSH secretion can vary independently of LH even though a single releasing hormone stimulates the production of both gonadotrophins simultaneously. This indicates that either testicular steroids influence LH and FSH secretion in different ways or that some other non-steroidal testicular factor can inhibit FSH secretion exclusively or predominantly. Evidence for the existence of such a testicular factor, inhibin as it has come to be known, has been extensively reviewed (Baker et al, 1976; Setchell et al, 1977; Davies et al, 1978; Main et al, 1979; Franchimont et al, 1979). By definition therefore, inhibin is a non-steroidal and non-androgenic substance produced in the seminiferous tubules which specifically or preferentially regulates FSH secretion.

In rodents, damage to the seminiferous epithelium induced by bilateral efferent duct ligation, irradiation, heat, cryptorchidism and anti-spermatogenic chemicals produced an elevation of FSH which is accompanied by hypersecretion of LH. This implied either that in the rat, seminiferous tubules play a part in the control of LH as well as FSH or that the Leydig cells are damaged at

the same time as the seminiferous epithelium or that Leydig cell functions are secondarily affected by local factors from the damaged tubules. Thus in rats, in which FSH and LH secretion are seldom dissociated, the significance of inhibin in relation to testicular steroids in the feedback control of FSH is not established. Rodents therefore are not satisfactory experimental models for study of the role of inhibin in humans. In the other subprimate species (sheep) in which the control of testicular functions has been intensively studied, hemi-castration, efferent duct ligation and local heating of the testes have produced small but selective increases in FSH (Walton et al, 1978; de Jong et al, 1980). Inter-pretation of these experiments however is complicated by the seasonal pattern of reproduction in this species.

The evidence for the existence of inhibin in the human male is probably stronger than in any other species. In infertile patients with disordered seminiferous tubular function, plasma FSH concentrations are elevated while LH and testosterone are usually normal or only slightly outwith the normal range. An inverse correlation between FSH and sperm count or germ cell population of the seminiferous epithelium in subfertile men has been demonstrated (see Section C). In men who underwent unilateral orchidectomy or chemotherapy for malignancies, monotrophic rise in FSH is observed (Bramble et al, 1975; Van Thiel et al, 1972). FSH-inhibiting activity was low or undetectable in the seminal plasma of men with severe seminiferous tubule

disease as indicated by azoospermia and elevated FSH (Franchimont, 1972; Scott & Burger, 1980). Using a radioimmunoassay, Vaze et al (1980) also demonstrated a lower concentration of "inhibin" in seminal plasma of oligospermic compared to normospermic men. So far no study has demonstrated a reciprocal relationship between circulating "inhibin" and FSH levels.

It is well recognized that under certain experimental conditions, exogenously administered testosterone can suppress FSH as well as LH secretion (for review see Baker et al, 1976). In most instances, pharmacological doses have been employed so that the physiological role of steroidal feedback control of FSH could not be assessed. Physiological concentrations of testosterone in the circulation produced by subcutaneous silastic implants do maintain normal levels of LH and FSH in the castrated rat and monkey (Moger, 1976; Davidson et al, 1976; Plant et al, 1978). However it has been demonstrated that the stable plasma concentrations achieved by testosterone implants may be more effective in suppressing LH and FSH secretion than the fluctuating levels seen in the intact animal (Dasmassa et al, 1976; Smith et al, 1977). The active and passive immunization of male animals against testosterone (Nieschlag et al, 1975; Gay & Kerlan, 1978) and the analogous inherited condition of testicular feminization (Naess et al, 1976) are associated with raised FSH as well as LH levels. These observations suggest that testosterone may well play a significant if not exclusive role in the physiological control of FSH as

well as LH secretion.

A preferential suppression of FSH secretion has been ascribed to oestrogens - especially oestradiol (Franchimont et al, 1975). In the castrated male monkey, physiological concentrations of testosterone and oestradiol produced by simultaneous silastic implants can maintain normal levels of LH and FSH after bilateral orchidectomy for up to twenty days (Resko et al, 1977). A synergistic role between testosterone and oestradiol in the feedback regulation of FSH was postulated. Other testicular steroids such as 5α-dihydrotestosterone, 17α-hydroxyprogesterone, 3α- and 3β-androstanediols did not show any selective suppression of FSH secretion (Swerdloff et al, 1973; Zanisi et al, 1973; Stewart-Bentley et al, 1974).

In man as well as experimental animals, selective lesions of the seminiferous tubules result in elevated plasma FSH concentrations which are considerably lower than that after surgical castration (Main et al, 1978). It is therefore possible that both testicular steroids and inhibin may contribute to the overall testicular feedback control of FSH secretion. This concept is supported by the elegant studies of Morris & Jackson (1978). Destruction of the germinal epithelium was induced by α chlorohydrin which produces obstructive cysts in the efferent duct and caput epididymis. This resulted in a selective rise of circulating FSH concentrations whose magnitude was only 30% of that seen after castration. In these same rats, destruction of

Leydig cells by ethane dimethane sulphonate caused further increases in FSH (and LH) indistinguishable from values seen after castration. The pattern of gonadotrophin response following the selective and consecutive removal of inhibin and androgen feedback suggests that inhibin probably plays a synergistic and minor role in the control of FSH secretion in the rat.

12.2.1 Sources of inhibin

Several biological fluids and testicular preparations have recently been shown to have inhibin-like activity based on the property of selective or preferential FSH suppression. The only human (male) source of inhibin so far detected is in seminal plasma (Franchimont et al, 1979; Vaze et al, 1979; Scott & Burger, 1980). In the latter two studies, there is evidence that the prostate, not the testes, contains the highest concentration of inhibin-like bio- or immunoactivity. In animals, inhibin activity has been detected in bull seminal plasma (Franchimont, 1975), ram rete testis fluid (Setchell & Sirinathsinghji, 1972), bovine spermatozoa extract (Lugaro et al, 1974) and ram testicular aqueous extract (Keogh et al, 1976). The cellular source of inhibin in the testes and testicular fluids is most probably the Sertoli cells (Steinberger & Steinberger, 1976; A. Steinberger, 1979; de Jong et al, 1978 & 1979).

Although historically inhibin is a testicular hormone elaborated in the seminiferous tubules, recent evidence suggests that a similar FSH-inhibitor can be detected in ovarian follicular fluids from several species

including man (reviewed by Setchell, 1980).

12.2.2 Physico-chemical properties of inhibin

Most investigators are now convinced that the active material in preparations containing inhibin-like activity is a peptide or glycoprotein molecule. Steroid concentrations in these preparations are negligible and charcoal adsorption does not alter their activity. However, there is considerable disagreement as to the molecular weight ranging from 5,000 to 160,000. No doubt this is related to the impure preparations used in most studies. On the other hand, inhibin may have a heterogeneous composition consisting of polymers or it may be associated with a carrier or a precursor molecule yielding smaller active fragments.

12.2.3 Detection and measurement of inhibin

The controversy surrounding inhibin is partly the result of the lack of sensitive methods of detection suitable for the measurement of this molecule in physiological fluids. To date, most available methods of inhibin measurement are bioassays based on the inhibition of pituitary FSH secretion. In vivo methods utilize intact or castrated animals or the inhibition of hCG augmented uterine weight gain in developing females. More sensitive and reliable are the in vitro techniques using either hemipituitary glands or pituitary cells in culture in which the basal or GnRH stimulated gonadotrophin levels are measured after several days incubation with the test material. However even the best of these in vitro bioassays lack the required sensitivity,

precision and reproducibility to study the physiological or pathological secretion of inhibin. Radioimmunoassays and radioligand assays of inhibin are at the preliminary stages only due to the lack of purified and homogeneous material for preparation of antibody and tracer. This will no doubt be more easily available in the near future and the wider study of the physiology of inhibin should be forthcoming. This topic was recently reviewed by Hudson (1979).

12.2.4 Mode of action of inhibin

There is little doubt that inhibin can exert negative effects on the basal synthesis and release of FSH and response to GnRH in pituitary gonadotropes. This has been demonstrated in both in vitro (Baker et al, 1976; Franchimont et al, 1978) and in vivo experiments (Blanc et al, 1978; Franchimont et al, 1977; Rush & Lipner, 1979). Surprisingly, in most in vivo studies, a prompt reduction in mean LH concentration and LH pulsatile secretion was observed during the infusion of inhibin. The explanation of this interesting observation is not clear. A latent period of 4-12 hours was observed before FSH suppression and this persists for up to 24 hours after the cessation of the infusion. This time course of FSH suppression favours the inhibition of pituitary FSH synthesis as the major mechanism of inhibin action.

The hypothalamic action of inhibin was suggested by the reduction of circulating FSH after injection of a bull spermatozoa extract into the third ventricle of male rats (Lugaro et al, 1974). In isolated rat hypothalami,

the GnRH content could be reduced after short term incubation with various inhibin-containing preparations (Demoulin et al, 1979). These preparations may also have direct effects on germ cells as indicated by the decreased incorporation of tritiated thymidine in type B spermatogonia from the developing rat (Franchimont et al, 1979).

For inhibin to exert its extratesticular effects, it has to be transported to the systemic circulation. Two possible routes for the exit of testicular inhibin have been proposed. The first is the caput epididymis where the rete testis fluid is actively reabsorbed (Setchell et al, 1977). The second, and probably more important, is from the basal surfaces of Sertoli cells where inhibin molecules can bypass the blood-testis barrier and find their way into the interstitial space (Setchell et al, 1977).

12.2.5 Physiological role of inhibin

Although evidence supporting the existence of inhibin in some species has grown rapidly in recent years, it remains difficult to define specific phsyiological role(s) for this elusive molecule. Experimental manipulations often do not and cannot completely isolate seminiferous tubular function from that of Leydig cells. This has resulted in a great deal of confusion in the interpretation of experimental data investigating the relative importance of steroidal and non-steroidal feedback control of FSH. Perhaps the most significant message from these studies is that normal function of the testis involves the intimate interaction between

Leydig, Sertoli and germ cells and disruption of the integrity of one will inevitably result in dysfunction of the other two testicular elements. It is therefore not surprising to find that the best studies in the literature usually suggest a synergistic relationship between inhibin, testosterone and oestradiol. However, unlike testosterone and oestradiol, the existence of inhibin in the circulation has so far not been confirmed. Thus the detection of inhibin in the systemic circulation and the demonstration of a reciprocal relationship with FSH remain the most important objectives in ascertaining an extratesticular physiological role for this putative hormone.

CHAPTER 13

PROLACTIN

Although the existence of prolactin in mammals as a lactogenic hormone has long been recognized, the isolation of human prolactin as an individual entity distinct from growth hormone and human placental lactogen was achieved only in 1971 (Lewis et al, 1971). This was soon followed by the development of specific radioimmunoassays (Hwang et al, 1971) enabling the rapid expansion of information concerning prolactin physiology and pathophysiology. Prolactin is a peptide hormone with 198 amino acid in a linear sequence (Shome & Parlow, 1977) synthesized by a specific pituitary cell type, the lactotropes, localized mainly to the postero-lateral wings of the pars distalis. The structure of human prolactin is similar to ovine, porcine and rat prolactin but distinct from that of growth hormone. The molecular weight of prolactin is 21,000 (Guyda, 1975). Several molecular forms can be recognized in pituitary and serum prolactin (Guyda, 1975; Suh & Frantz, 1974). The major fraction in the circulation is the monomeric form constituting 75-80% of total prolactin. A larger molecule MW 40,000 'big' prolactin, is also present in the circulation to the extent of 10-15%. Occasionally, a very high molecular weight form, 'big big' prolactin, can be found in the circulation in pregnancy and in some cases of prolactin-secreting pituitary adenomas.

Early studies on prolactin were performed on pigeon

crop sac bioassay and the induction of lactational response in pseudopregnant rabbits. Apart from the low sensitivity, these bioassays do not distinguish between the lactogenic effects of primate growth hormone and human placental lactogen from that of prolactin. This lack of specificity presented great obstacles to the purification and separation of these hormones in early studies. The immunological specificity of these hormones finally permitted their separation and the development of radioimmunoassay followed shortly.

13.1 Control of prolactin secretion

Prolactin, unlike gonadotrophins, is controlled by both inhibitory and stimulatory influences from the hypothalamus. In mammalian species, prolactin synthesis and release is predominantly under tonic hypothalamic inhibition (Everett, 1954). Using a sensitive paired pituitary incubation technique, Sharr & Clemens (1974) showed that physiological amounts of dopamine inhibited the release of prolactin from pituitaries in vitro. Intraventricular infusion of dopamine can suppress prolactin secretion in the intact rat (Kamberi et al, 1971). A porcine hypothalamic preparation prepared by Takahara et al (1974) inhibited prolactin secretion when infused into the hypophyseal portal system in rats in a way closely correlated with the dopamine content. The demonstration of dopamine receptors in the anterior pituitary provides further evidence to support the physiological role of this catecholamine (Brown et al,

1976). Although the dopamine concentrations in the portal vessels exceed that in the systemic circulation, plasma prolactin levels are not always appropriately correlated with dopamine in portal blood (Ben-Jonathan et al, 1977; Gibbs & Neill, 1978). Two explanations have been suggested to account for this apparent discrepancy. Dopamine may not be the only prolactin inhibiting factor (PIF) in the hypothalamus and other compounds like GABA may also have PIF activity (Schally et al, 1977). Alternatively, a prolactin releasing factor (PRF) may antagonize the influence of PIF so that plasma prolactin concentration is determined by the balance between the two opposing factors. More remains to be learned about the physiological mechanisms controlling prolactin secretion.

The evidence for the existence of a prolactin releasing factor is limited. Although many spontaneously occurring peptides (β endorphin, neurotensin, substance P, bombesin) can stimulate prolactin release, they act on the brain to modify PIF release rather than stimulate the pituitary directly (Meites et al, 1979 for review). Thyrotrophin releasing hormone (TRH) is a potent stimulator of prolactin release (Tashjian et al, 1971). However a physiological role for TRH in the control of prolactin is unlikely since the secretion of these two hormones can be clearly dissociated under various physiological conditions, e.g. pregnancy and lactation. Recent studies suggest that vasoactive intestinal polypeptide (VIP) may fulfil the role of physiological PRF

(Said et al, 1978; Samson et al, 1978). This will require further confirmation.

A short-loop feedback of prolactin secretion may exist. This is thought to be mediated via the hypothalamus and involves alterations in the metabolism and turnover of dopamine (Fuxe et al, 1977) in the tuberoinfundibular neurones. This may furnish a basis whereby elevated prolactin levels could alter the control of gonadotrophin and GnRH by influencing the common monoamine metabolism in the brain (Neil, 1974). Oestrogens stimulate prolactin secretion via actions on the hypothalamus and pituitary (Labrie et al, 1978). Prolonged oestrogen stimulation in rats gives rise to proliferation of pituitary lactotropes raising the possibility of induction of prolactinoma in women exposed to the contraceptive pill (Lloyd et al, 1975).

As is the case for other pituitary hormones, prolactin is secreted episodically with intervals of 20-30 minutes. This is in keeping with the short circulating half-life of this hormone. A distinct diurnal variation in prolactin secretion exists (Nokin et al, 1972; Sassin et al, 1973; Parker et al, 1973), and maximal concentrations occur some six to eight hours after sleep onset. A variety of stimuli can lead to increased prolactin secretion. In the female, pregnancy and lactation are well recognized to be associated with increased prolactin in the circulation. Other stimuli include physical and psychological stress, sexual intercourse, nipple manipulation and changes in plasma

osmolality. Seasonal changes in light and temperature can also affect prolactin secretion in some species, e.g. hamster and sheep.

13.2 Mode of action and biological effects of prolactin

Although over one hundred physiological actions have been ascribed to prolactin (Nicholl et al, 1977), only its role in lactation and reproduction in humans has been established. It is beyond the scope of this chapter to discuss the role of prolactin in lactation and ovarian steroidogenesis. In the male, interests in the effect of prolactin on reproductive function are comparatively recent and poorly documented. The first relevant study was the demonstration of a synergistic effect between LH and prolactin in restoring and maintaining accessory gland weight and spermatogenesis in hypophysectomized rats (Woods & Simpson, 1961). Using the same experimental model, subsequent studies showed that prolactin alone was ineffective and did not potentiate the effect of testosterone on spermatogenesis (Bartke, 1971a; Hafiez et al, 1972). The effect of LH on Leydig cell steroidogenesis in hypophysectomized rats was increased by prolactin. The action of prolactin on Leydig cells was supported by the demonstration of specific receptor binding for this hormone in the interstitium of the rat testis (Aragona et al, 1977; Charreau et al, 1977). Although prolactin is detectable in human seminal plasma (Sheth et al, 1975) its role in spermatogenesis or maturation of sperms has not been convincingly demonstrated.

Several possible mechanisms for the testicular action of prolactin have been postulated. Prolactin may increase the concentration of testicular LH receptors (Zipf et al, 1978; Purvis et al, 1979) or increase substrate availability for steroidogenesis (Bartke, 1969, 1971b) or enhance the activity of testicular 17β-hydroxysteroid dehydrogenase (Musto et al, 1972) and 3β-hydroxysteroid dehydrogenase (Hafiez et al, 1971). The relative importance and interrelationships between these postulated mechanisms of action remain to be established. In considering the biological effects and mechanism of action of prolactin, the most striking feature is the interaction with other hormones to achieve the observed target response. Especially in the male, prolactin action seems to be a permissive rather than a critical one based on modification of the interaction of more specific trophic hormones and their receptors in target organs.

Reproductive functions in male patients with hyperprolactinaemia have not been studied extensively. Erectile impotence and diminished sex drive seem to be the most prominent clinical symptoms which are out of keeping with the minimally depressed circulating androgen concentrations (Thorner et al, 1977; Segal et al, 1976; Carter et al, 1978; Franks et al, 1978; Peillon et al, 1979). Neither the evidence for Leydig cell dysfunction nor that impotence is directly related to hyperprolactinaemia rather than testosterone deficiency can be regarded as conclusive. There is a great need for basic controlled endocrine, pathophysiological and fertility studies in male patients with hyperprolactinaemia.

SECTION B

STUDIES ON DELAYED PUBERTY

CHAPTER 1

INTRODUCTION

1.1 Definition of puberty

Puberty is generally referred to as the phase of development during which reproductive capacity is attained. In all mammals, the reproductive system is the last organ system to mature. Puberty thus represents the climax of an individual's overall growth and development, a biological watershed linking immaturity to maturity, which henceforth gives way to the process of ageing. With the better understanding of the hormonal and genetical mechanisms of prenatal sexual differentiation and development (Jost, 1972; Ohno, 1976; Wilson, 1980) puberty has come to be regarded as the final stages of a continuous series of genetically-programmed and hormone-mediated events spanning from conception to adulthood (Grumbach, 1980) which culminates in the optimal function of the hypothalamic-pituitary-testicular axis.

1.2 Physical changes of puberty

In animals, puberty can be temporally defined by the time of completion of spermatogenesis or when first ovulation occurs. In humans, however, these parameters cannot be precisely delineated on clinical examination or even by laboratory investigation. Puberty in man has therefore traditionally been equated with a series of

developmental changes in the secondary sexual characteristics. Indeed, the term puberty is derived from the word pubes (Greek - hair) denoting the stage when certain areas of the body become covered with hair which characterizes sexual maturity. The physical changes of puberty involve both sexual and non sexual organs and can be grouped into: (1) a general increase in the growth rate of the skeleton, muscle and viscera known as the adolescent growth spurt; (2) sex-specific changes in circulatory, respiratory, musculo-skeletal systems and the quantity and distribution of body fat, leading to an enhancement of sexual dimorphism in physical strength, endurance and body proportions; (3) the development of the gonads, reproductive tracts and secondary sexual characteristics (Tanner, 1974; Marshall & Tanner, 1974; Forest, 1981). It is obvious that puberty is not a single isolated event but a conglomerate of physical, psychological and social changes centred around the maturation of hypothalamic, pituitary and gonadal functions. These gradual changes develop concurrently over a period of years but are variably related to each other in sequence and tempo and also to chronological and skeletal age (Marshall & Tanner, 1974). Thus, although all the physical changes in puberty can ultimately be attributed to gonadal hormone actions (Short, 1980), each of them merit individual consideration independently (Tanner, 1974). A detailed account of the adolescent physical growth and metabolic changes is beyond the scope of this chapter, which deals primarily with the hormonal

aspects of puberty. It is sufficient here to mention that the adolescent growth spurt is a well-defined period during which height increase reaches a maximum (about 10 cm/yr). This occurs at a mean age of 14.06 ± 0.14 in boys (Tanner, 1974) but some two years earlier in girls. The growth spurt often provides a better biological reference point during puberty than either chronological or bone age against which other parameters can be aligned.

The pubertal changes in the reproductive system have been systematically studied (Schonfeld & Beebe, 1942; Schonfeld, 1943; Reynolds & Wines, 1951; Tanner, 1962; Zachmann et al, 1974). The most widely accepted scheme for the rating of physical changes in the reproductive system is that proposed by Tanner (1962). According to this classification, male puberty is divided into five clinical stages by the progressive changes in external genitalia and pubic hair.

1.2.1 Genital (G) stages

G1 - Size and general appearance of the testes, scrotum and penis are about the same as in early childhood - prepubertal.

G2 - The scrotum and testes have enlarged and there is some reddening and change in texture of scrotal skin.

G3 - The penis has increased in length and to a lesser extent in breadth. There is a further growth of the testes and scrotum.

G4 - The length and breadth of the penis have increased further and the glans has developed. Testes and scrotum have further enlarged with darkening of the

scrotal skin.

G5 - Genitalia are adult in shape and size.

1.2.2 Pubic Hair (PH) stages

PH1 - No true pubic hair. Only vellus hair present.

PH2 - There is a sparse growth of long straight or only slightly curled and lightly pigmented hair chiefly at the base of the penis.

PH3 - The hair spreads sparsely over the junction of the pubes and is considerably darker, coarser and more curled.

PH4 - The hair is now adult in type but covers a considerably smaller area than adults. There is no spread to the medial surface of the thighs.

PH5 - Hair is distributed as an inverse triangle as in the adult female. It has spread to the medial surface of the thighs but not up the linea alba or elsewhere above the base of the triangle.

Most men's pubic hair develops more extensively than stage PH5 but this occurs gradually over the decade after completion of puberty, and the extent of this further development is subject to individual and racial variations.

1.2.3 Testis size

Testicular growth during puberty can be monitored conveniently by the increasing testicular volume or diameter, providing a useful clinical index of seminiferous tubular size and function. The assessment of testicular size is most commonly achieved by visual

Puberty stage	Testis Size (ml) Mean (SD) Zachmann et al 1972	Waaler 1974	Burr et al 1970	August et al 1972	Average from four studies
1	2.5 (1.7)	1.5 (0.5)	2.77 (0.62)	1.7 (0.85)	2
2	3.4 (2.3)	4.2 (1.3)	4.80 (1.71)	4.3 (2.16)	4
3	9.1 (5.3)	10 (2.3)	8.99 (2.12)	8.2 (1.94)	9
4	11.8 (5.8)	11 (3.8)	13.16 (2.09)	10.4 (2.32)	12
5	14.0 (4.6)	15 (3.0)	-	-	15

Table 1.1 Comparison of testicular sizes in pubertal subjects in four separate studies.

or tactile comparison of volume with a series of standard elipsoids first used by Schonfeld & Beebe (1942) and modified and popularized by Prader (1966). Others have chosen to use calipers to measure testicular length and width in order to calculate the volume (August et al, 1972; Waaler et al, 1974). The results of pubertal testicular size increase have been validated (Schonfeld & Beebe, 1942) and values from different studies are comparable (Table 1.1). Considerable overlap in mean and individual testicular volumes was observed in all stages of puberty in these cross-sectional studies. A consistent finding was that the prepubertal testicular volume was usually 1-2 ml while volumes of 3-4 ml or more were generally compatible with early or impending sexual maturation. In their classic paper, Schonfeld & Beebe (1942) were particularly struck by the sharp distinction between prepubertal and pubertal testicular volumes. They consequently recommended the measurement of testicular volume as a valuable clinical criterion for determining the onset of puberty. However, once puberty has started, isolated estimation of testicular volume on its own may not be particularly meaningful due to the wide range of sizes at each stage of puberty. Serial measurements of testicular volume combined with genital and pubic hair ratings in individual pubertal subjects are useful in assessing the progress of sexual development.

1.2.4 Onset of spermatogenesis

An important aspect of male puberty which is difficult to assess in humans is the time at which sperm

production first occurs. This after all is one of the hallmarks of sexual maturity (see definition of puberty). Earlier histological studies of the testes were difficult to interpret due to the lack of clinical and hormonal data (Sniffen, 1950; Charny et al, 1952; Albert et al, 1953; Mancini et al, 1960; Vilar, 1970). Not surprisingly, these reports on the sequence of maturation of various cell types in the seminiferous tubules were conflicting. Recently, the presence of sperms in the urine (spermaturia) has been used as an indicator of mature spermatogenesis in puberty (Richardson & Short, 1978). It was demonstrated that the mean age at which spermaturia was first detected was 13.3 $\pm$ 0.12 years. Although no genital examination was carried out, it could be concluded that spermatogenesis is established before most of the androgen-dependent secondary sexual characteristics. Even though spermaturia cannot be equated with fertility, this still contrasts sharply with female puberty where menarche occurs in the later stages and ovulatory cycles do not regularly occur until much later (Tanner, 1962; Billewicz et al, 1980).

1.2.5 Other maturational markers of puberty

Hair on the face, axilla, trunk and extremities and temporal recession appear about the same time in late puberty - around G4, PH4 or two years after the onset of pubic hair growth (Tanner, 1974; Forest, 1981). However, these features are so variable in extent that they are of little value as indices of puberty. Growth of facial and

body hair is seldom complete until well after the completion of puberty.

Enlargement of the thyroid and cricoid cartileges and the lengthening of the vocal cords from growth of the larynx are responsible for breaking or deepening of the voice (Daw, 1970). This usually begins at the time of peak height velocity in mid-puberty or around age 13.5 years (Karlberg et al, 1976) and is completed at the end of puberty. Again this has limited clinical value.

Peak height velocity in boys occurs between stages G4-G5. This is markedly different from girls whose peak height velocity occurs much earlier - between breast stages 2-3 or nearly two years earlier than boys (Marshall & Tanner, 1969).

The occurrence of first ejaculation was closely correlated to the appearance of pubic hair according to Kinsey et al (1948). Spontaneous nocturnal emission in this study occurred a year later.

1.2.6 Variation in pattern of puberty

There are wide variations in age at which boys reach the individual stages of genital and pubic hair development (Marshall & Tanner, 1970). It follows that stages of sexual development are poorly correlated with chronological age. Although often assumed not to be the case, the work of Marshall (1974) has shown that skeletal age is equally variable when correlated with pubertal stages. The average age in British boys at the onset of puberty (G2) is 11.64 $\pm$ 1.07 years with a range of 9.5-13.5 at 95% confidence limit.

Pubic hair and genital development are not very closely correlated with each other (Marshall & Tanner, 1970). This is why puberty ratings should be designated by G and PH stages separately. It is however true that genital development is usually in advance of pubic hair in the present scheme.

The duration of the various stages and hence the total period of pubertal development also display considerable variability (Marshall & Tanner, 1970). Some boys may remain in stage G2 for up to 2½ years while others may complete the entire genital development in under this time. The mean duration between G2 and G5 in boys is 3.05 years (1.86-4.72, 95% confidence limit).

1.3 Hormonal changes of puberty

A large body of physiological studies has established that the interaction between the gonads and the hypothalamic-pituitary unit is operative from early childhood (Critchlow & Bar-Sela, 1966; Donovan, 1963). Bioassays for gonadotrophins provided further supportive evidence with the demonstration of both LH and FSH activities in the urine of normal prepubertal children (Brown, 1958). However, the insensitivity of bioassays and the inaccuracy of urine collections militated against the precise quantitation of gonadotrophin and steroid secretion. The availability of sensitive radio-ligand assays in small quantities of biological fluids has enabled investigators to study the interrelationships between the gonads and pituitary in childhood and puberty.

The principal questions the early studies attempted to answer were: (1) at what stage of development do gonadotrophins begin to rise towards adult levels; (2) what is the subsequent time course once the increase has begun; (3) what is the temporal relationship between changes in concentrations of gonadal steroids, gonadotrophins and the development of physical changes in puberty; (4) does the above information help to formulate the physiological mechanisms underlying the initiation and progression of puberty.

To a large extent, the first two questions have been satisfactorily answered despite some discrepancies between different studies. The latter two more complex questions have still to be comprehensively tackled. Designs in the earlier studies were subject to various criticisms. Hormonal values during puberty are less meaningful when correlated only to chronological age which is widely variable at each stage of development. Although the stages of pubertal development are more informative, the same variability in timing of onset and duration seriously undermined the value of these cross-sectional studies of puberty which tended to obscure the changes across time during sexual maturation. As pointed out by Tanner (1962), it is this rate of change in growth and hormone function in a given time illustrated by longitudinal studies which are of most value in unravelling the complex interrelationships and mechanisms of puberty. Other confounding factors in the study of hormonal changes in puberty are the episodic

nature of hormone secretion which becomes detectable for the first time during normal puberty and the diurnal patterns of hormone secretion in relation to sleep in early and mid-puberty. In the ensuing summary of hormonal patterns at different stages of puberty based mainly on single sample day-time cross-sectional studies, it is useful to bear these reservations in mind.

1.3.1 Peripubertal basal hormone patterns

The information summarized from the large number of studies (Blizzard et al, 1972; August et al, 1972; Burr et al, 1970; Winter & Faiman, 1972; Knorr et al, 1974; Gupta, 1975; Sizonenko et al, 1975) is expectedly heterogeneous but a few general trends can be demonstrated. Luteinizing hormone, FSH, testosterone, dihydrotestosterone, oestrone and adrenal andorgens all progressively increased from very low levels characteristic of early childhood to adult levels during puberty. However the time of onset, rate of increase and the extent of the increments varied considerably in different studies. Nevertheless, most studies would agree that LH and testosterone usually increased in parallel with LH changes usually preceding testosterone. Plasma testosterone rise occurred after the first signs of testicular enlargement. FSH increased early in puberty in most studies and a good correlation has been obtained with increase in testicular size. The greatest increase in testosterone occurred in stages PH2-3 while the levels continued to rise even after stage 5. LH increments were greatest during early puberty and then plateaued out after

mid-puberty, even though testosterone continued to increase. FSH increments were smaller in magnitude than LH and reached adult levels earlier. Although statistically significant increases in mean LH and FSH levels were seen in all studies with advancing age or maturation, there was substantial overlap amongst individuals in different pubertal stages.

Dihydrotestosterone and 3α-androstanediol increased in parallel with testosterone except during the earliest stages of puberty when there was a relative, but transient, abundance of the 5α-reduced androgens compared with the prepubertal and adult periods.

A modest increase in oestradiol and oestrone can be observed in boys during puberty (Bidlingmaier et al, 1973; Angsusingha et al, 1974; Gupta et al, 1975; Lee & Migeon, 1975; Ducharme et al, 1976; Thorner et al, 1977; Large & Anderson, 1979).

Plasma concentrations of adrenal androgens, dehydroepiandrosterone (DHEA) and its sulphate (DHAS) and androstenedione (Δ) started to rise from at least two years before the onset of gonadal development (Lee & Migeon, 1975; Hopper & Yen, 1975; Sizonenko & Paunier, 1975; Ducharme et al, 1976; Korth-Schutz et al, 1976a & b; Sizonenko et al, 1976; de Peretti et al, 1976 & 1978; Parker et al, 1978) and continued to do so until adult levels were reached by about stage 3-4 of gonadal puberty. The onset of increasing adrenal androgen secretion around the age of seven years was associated with the increase in relative size of the zona reticularis in the adrenal

(Dhom, 1973) and has been called the adrenarche to distinguish it from gonadarche which followed later. Although adrenarche and gonadarche are normally temporally linked, they can be dissociated from each other under pathological conditions without affecting the timing or function of the other (Sklar et al, 1980). Thus normal gonadarche can occur in the absence of adrenarche - for example in Addison's disease - and normal adrenarche can occur without gonadarche - for example hypogonadism. It seems unlikely therefore that adrenal androgens exert a major influence on the timing of gonadal pubertal onset (Cutler & Loriaux, 1980).

The foetal and postnatal adrenal can produce high levels of adrenal androgens comparable to the adult (Forest et al, 1978). The rapid fall in peripheral plasma concentration of these hormones is paralleled by the involution of the foetal zone of the adrenal glands (Beck, 1971). The low levels of adrenal androgens are maintained in early childhood until the abrupt resurgence of secretory activity in the zona reticularis at around seven years. This ontogenic pattern of adrenal androgen production bears a remarkable resemblance to that of gonadal function (Forest et al, 1978) suggesting that CNS factors may regulate the (?pituitary) trophic control of the adrenal in a similar way to the neuroendocrine control of gonadal puberty. The indirect clinical evidence supporting the existence of a pituitary factor acting in synergy with ACTH to control adrenal androgen production was recently reviewed (Grumbach et al, 1978).

Despite the many similarities between gonadarche and adrenarche, our present understanding of the mechanism and control of the latter lags considerably behind that of gonadal puberty.

Although plasma oestradiol and oestrone concentrations show a small but significant rise during puberty (Bidlingmaier et al, 1973; Angsusingha et al, 1974; Gupta et al, 1975; Lee & Migeon, 1975; Baker et al, 1976; Thorner et al, 1977), the mean prolactin concentration is not different between boys and adult males (Guyda & Frieson, 1973; Lee et al, 1974; Ehara et al, 1975; Aubert et al, 1977; Thorner et al, 1977). Recent studies have also confirmed the lack of pubertal change in prolactin secretion in 24 hour profiles (Finkelstein et al, 1978; Large et al, 1980; Beck & Wuttke, 1980).

SHBG binding capacity in the plasma of children of both sexes are similar but higher than adults (August et al, 1969). During puberty SHBG falls in both boys and girls but to a greater extent in males whose SHBG level is approximately 50% that of the adult females. This amplifies the relative increase in unbound testosterone in the male during puberty.

1.3.2 Pulsatile gonadotrophin secretion in puberty

The adult male's pattern of gonadotrophin secretion is characterized by nine to twelve secretory episodes every twenty-four hours superimposed on a stable baseline (Boyar et al, 1972a; Santen & Bardin, 1973). The episodic gonadotrophin secretion probably reflects the pulsatile release of GnRH from the median eminence into

the hypophyseal portal circulation (Carmel et al, 1976; Eskay et al, 1977; Neil et al, 1977). In prepubertal children, the very low peripheral plasma basal concentrations of gonadotrophins are associated with either undetectable or minimal pulsatile fluctuations, which first become apparent just prior to the clinical onset of puberty (Judd et al, 1977; Boyar, 1978). This important marker of the activation of the hypothalamus at the onset of puberty has been construed as evidence supporting the prime importance of neuroendocrine mechanisms of pubertal onset. Moreover, this concept is further supported by the finding that episodic gonadotrophin secretion in the early stages of puberty is only observed during sleep, either at night or daytime (Boyar et al, 1972b & 1974a; Kapen et al, 1974). These exciting data, obtained by the combined use of frequent blood sampling, sensitive RIA methods and polygraphic sleep recording, entirely reshaped the perspective of hormone secretory dynamics during puberty and attributed an important biological role to sleep, a functional neuronal activity state, in normal sexual development. As puberty advances, episodic gonadotrophin secretion becomes evident during the waking hours and at maturity, the uniform pattern with no sleep-wake differential in adults is established (Weitzman et al, 1975). FSH secretion is also augmented synchronously with nocturnal sleep in pubertal children although the pulsatile pattern is less clearly defined than LH due to the longer circulating half-life of FSH (Yen et al, 1970).

The importance of sleep-entrained LH secretion in

sexual development is further substantiated by the demonstration of a close temporal relationship between testosterone and LH secretion during nocturnal and diurnal sleep (Boyar et al, 1974; Judd et al, 1974). These data also add weight to the argument against the critical role of shifting negative-feedback threshold of gonadal steroids as the mechanism for pubertal initiation. This, together with the findings that patients with gonadal dysgenesis have a qualitatively similar pattern of pubertal sleep-related gonadotrophin secretion (Boyar et al, 1973) suggest that central neuroendocrine mechanisms must be primarily responsible for the initiation of hypothalamic GnRH secretion at the onset of puberty. The nature of the mechanism(s) and their relationship to sleep is unknown but neurotransmitter monoamines are likely to be intimately involved (Jouvet et al, 1963).

1.3.3 Urinary gonadotrophins

Radioimmunoassay of excreted gonadotrophins in urine has been used to detect changes, especially during sleep, in rate of gonadotrophin secretion during puberty. Although good correlations between mean plasma concentrations and 24-hour urinary gonadotrophin excretion have been obtained (Kulin et al, 1975 & 1976) the data with shorter timed urine collections are inconsistent (Beitins et al, 1976; Urban et al, 1979; Chipman et al, 1981). Furthermore, reservations concerning the variability in bio- and immuno-activity between plasma and urinary gonadotrophins (Raiti et al, 1975; Ascoli & Puett, 1976)

and that produced by different extraction procedures (Blizzard et al, 1972) render the validity of urinary measurements questionable.

1.3.4 Pituitary responsiveness in puberty

The pubertal rise in plasma gonadotrophin concentrations may be induced by increased GnRH stimulation but enhanced pituitary responsiveness with advancing sexual maturity can also contribute a significant part. Studies using exogenous GnRH in single bolus stimulation (Kastin et al, 1972; Job et al, 1972; Roth et al, 1972; Grumbach et al, 1974) and continuous infusion (de Lange et al, 1974; Reiter et al, 1976; Huseman & Kelch, 1978) confirmed that the LH, and to a more variable extent FSH, responses increased with pubertal maturation. There is thus an expansion of the pituitary reserve of gonadotrophins during sexual development which may result from the priming effects of hypothalamic GnRH stimulation (Grumbach et al, 1974; Mortimer et al, 1974; Reiter et al, 1975 & 1976; Boyar et al, 1976). With prolonged GnRH stimulation by continuous infusion, there is also evidence that the presence of an immediately releasable and a reserve pool of LH can be elicited in adult and pubertal but not prepubertal subjects (Reiter et al, 1976).

1.3.5 Gonadal responsiveness in puberty

During puberty, the two to three-fold rise in plasma LH is associated with a twenty-fold increase in testosterone secretion. This indicates that an amplification step also occurs at the gonadal level. This is confirmed

by the enhanced testosterone response to hCG stimulation at puberty (Winter et al, 1972; Schöller et al, 1975). The mechanism of this augmented testicular response to trophic stimuli at puberty may take several forms. Hyperplasia or hypertrophy of the Leydig cells (Hooker, 1970; Christensen & Peacock, 1980), increased LH receptor numbers (Odell & Swerdloff, 1976), alterations in steroidogenic or metabolic enzyme activities within the Leydig cells (Frasier et al, 1969; Strickland et al, 1970; Payne & Jaffe, 1975) could all contribute to the gonadal component of pubertal maturation represented by the increased testosterone production (and onset of spermatogenesis). The roles of FSH and prolactin in these functional changes of Leydig cells in puberty have recently been reviewed by Bartke (1978). Although the evidence for a significant contribution of these two pituitary hormones in animals are strong, data in humans are scanty and unconvincing.

The foregoing review described a hormonal pattern compatible with a sequential maturation of the hypothalamus, pituitary and testis which coincides with the progressive physical changes of puberty. It can be postulated that the central neural signals at the onset of puberty are translated or integrated by the hypothalamus into discrete pulses of GnRH secretion. These endocrine signals are then magnified at the level of the pituitary and again at the gonads so that the original neural signals have been amplified several hundred or thousand fold. This forward or open-loop system should be

considered alongside the alternative closed-loop system whereby the role of steroid negative-feedback sensitivity adjustments are of overriding importance. It would be interesting to consider these two concepts of hypothalamic-pituitary-testicular dynamic control in the light of the above descriptive data to see if a physiological mechanism for the initiation and progression of puberty can be formulated.

1.4 The mechanism of puberty

The very specific temporal and physiological conditions under which sexual function is activated have led to the supposition that puberty inducing mechanisms and timing devices must exist. However, puberty is also part of an ontogenic sequence of gonadal function which begins in foetal life. Thus any proposed mechanisms have not only to explain the peripubertal hormonal events but also to take into account the earlier changes in the hypothalamic-pituitary-testicular functions.

1.4.1 Ontogeny of gonadal function in foetal life and early childhood

The foetal hypothalamus and pituitary are not only active towards the end of the first trimester but are functional at the adult level quantitatively near mid-gestation when LH and FSH concentrations reach adult castrate levels (Faiman et al, 1981). From this point until term, oestradiol, presumably from the placenta (Siiteri & MacDonald, 1966), gradually increases while gonadotrophins and testosterone fall towards very low

levels. This indicates the acquisition of oestrogen negative feedback mechanisms. At the end of the first post-natal week, coincidental with the fall in circulating placental hCG and oestrogen, LH and FSH concentrations rise sharply with a concomitant increase in testosterone. From six months to two years of age, serum gonadotrophins and testosterone concentrations fall again to very low levels and remain so until the onset of puberty. It is believed that the mechanism(s) which restrain the hypothalamic-pituitary-testicular function and hence pubertal development for the next decade become established at this stage (between one to two years of age) in early childhood.

1.4.2 Reactivation of the hypothalamus

Immature gonads are able to secrete steroids in response to exogenous gonadotrophins (Price & Ortiz, 1944). The experiments of Harris & Jacobson (1952) showed that immature pituitaries transplanted under the median eminence of adult hypophysectomised female rats can maintain full ovarian functions. Recently, complete pubertal development has been successfully induced in immature female monkeys bearing lesions in the hypothalamus by exogenous pulsatile GnRH treatment (Wildt et al, 1980). It has also been demonstrated that immature rat hypothalamus contains considerable amounts of GnRH (Araki et al, 1975) with a functional portal system capable of delivering the releasing hormone to the pituitary (Halasz et al, 1972). These and other experimental studies

summarized in Donovan & Van der Werff ten Bosch (1965) established beyond doubt that the reproductive tract, the gonads, the pituitary and hypothalamus are all in a complete state of readiness to assume adult functions long before the normal time of puberty. The only qualification to this statement is the evidence that germinal tissues in most mammals have to undergo specific developmental changes before becoming responsive to gonadotrophins (Woods & Simpson, 1961; Critchlow & Bar-Sela, 1966). Otherwise all that is required for the onset of puberty is the reactivation or de-repression of hypothalamic GnRH secretion - the reversal of those mechanisms which suppressed the hypothalamus during the past eight to ten years. Two different theories have been put forward to explain the nature of this pre-pubertal restraint on hypothalamic GnRH secretion: (1) a highly sensitive negative feedback mechanism effected by gonadal steroids - closed-loop system - and (2) extra-hypothalamic inhibitory mechanisms either within or outwith the CNS - open-loop system.

1.4.3 Closed-loop system (Gonadostat Hypothesis)

The existence of an intact feedback relationship between the gonads and pituitary/hypothalamus was established by numerous physiological studies in the rat (Raimirez, 1973, for review). The greater sensitivity of the hypothalamic-pituitary unit to feedback inhibition of gonadal steroids before than after sexual maturation was also first demonstrated in the rat (Byrnes & Meyer, 1951;

Raimirez & McCann, 1963). These observations formed the experimental basis of the gonadostat hypothesis (Donovan & Van der Werff Ten Bosch, 1959; McCann & Raimirez, 1964) which postulated that a highly sensitive gonadostat is responsible for the suppression of gonadotrophins in prepuberty even in the presence of very low levels of gonadal steroids. At the onset of puberty, a reduction in the sensitivity of the gonadostat causes the increase in GnRH and gonadotrophin secretion which in turn stimulates gonadal functions. This downward shift in gonadostat sensitivity eventually stabilizes after adult concentrations of gonadotrophins and gonadal steroids are achieved and a new equilibrium is established. Support for this hypothesis was obtained in humans when urinary gonadotrophin secretion rate in prepubertal children were shown to be five to ten times more sensitive than adults to the inhibitory effect of exogenous ethinyloestradiol (Kelch et al, 1972 & 1973). A similar decrease in sensitivity to the oestradiol inhibitory feedback action on plasma LH has been demonstrated in the ovariectomized female lamb during puberty (Ryan & Foster, 1979). One criticism of some of the experiments in castrated rats and sheep centres around the fact that the castration response itself is age-dependent so that tests of feedback sensitivity in castrated may not be comparable to that in intact animals (Dierschke et al, 1974). An additional drawback of the gonadostat hypothesis is that it cannot easily explain the changes in hypothalamic-pituitary-testicular function

in the intra-uterine and early childhood phases without there being repeated upward and downward shifts in the gonadostat set point. Furthermore, in prepubertal agonadal girls, despite the higher basal concentrations of LH and FSH, the pattern of gonadotrophins is similar to normal intact children with the fall in concentration around age one to two years and subsequent rise at the expected age of puberty (Conte et al, 1975). The pituitary response to GnRH stimulation in these agonadal children also paralleled the diphasic pattern of basal gonadotrophins (Conte et al, 1980). The sleep-entrained episodic enhancement of gonadotrophin secretion typical of normal puberty is also present in subjects with gonadal dysgenesis at the time of puberty (Boyar et al, 1973). These interesting clinical studies clearly demonstrated that changes in patterns of gonadotrophin secretion in childhood and puberty can occur independently of gonadal steroids without the need to invoke negative feedback mechanisms.

1.4.4 Open-loop system (Neuroendocrine Hypothesis)

The existence of CNS mediated mechanisms capable of inhibiting the activities of GnRH-secreting hypothalamic neurones has been postulated since the recognition of clinical associations between precocious puberty and lesions in and around the hypothalamus and pineal (Thamdrup, 1961; Donovan & Van der Werff Ten Bosch, 1965). Presumably, these lesions can interrupt the inhibitory neuronal input or exert direct irritative effects on the

hypothalamus (Ruf et al, 1974) causing premature increase in GnRH output. Numerous studies attempting to identify and localize hypothalamic and extra-hypothalamic structures involved in the control of puberty have produced conflicting results. Essentially, the anterior and posterior hypothalamus, amygdala, hippocampus and pineal have all been implicated and perhaps the best explanation of these results is that the neuronal control of the hypothalamus depends on the balance between excitatory and inhibitory inputs from some or all of these areas whose roles may change with age of the animal (Gorski, 1974; Ramaley, 1980). In recent years, interest in the pineal has intensified since the recognition that pineal hormones such as melatonin and arginine vasotocin may have anti-gonadotrophic or anti-gonadal effects (Pavel & Petrescu, 1966; Reiter, 1974; Reiter & Vaughan; 1977; Benson, 1978). The unconfirmed data of Silman et al (1979) described a dramatic fall in circulating melatonin levels in 51 children aged 11½-14 years shortly before the onset of puberty. It is of further interest that the pineal gland evolved from a primitive photoreceptor organ and mediates several rhythmic biological functions including the seasonal reproductive cycles in several species - ram, hamster, ferret and vole (Licoln, 1981). That the sleep-wake cycle in man is intimately involved in the initiation of gonadotrophin secretion at the onset of puberty is in concordance with the experimental data supporting the prime importance of CNS modulation of hypothalamic

function. However, the major drawback to the CNS-disinhibition hypothesis is our lack of basic understanding of the nature and site of the neural or neurohumoral mechanisms involved. Despite this, the evidence for the existence of such mechanisms is convincing and the open-loop neural activation concept of puberty is gaining wide acceptance. A change in feedback sensitivity of the pituitary and hypothalamus may well exist concurrently as a secondary phenomenon to the more basic mechanisms of CNS activation of the hypothalamus. Clearly, our present understanding of the complex events and mechanisms of puberty is inadequate and more basic information is required.

1.5 Timing of pubertal onset

Although the gradual sequence of events during sexual maturation is genetically programmed, the timing of the initiation of puberty is closely linked to the external and internal environmental factors through modification of the neuroendocrine mechanisms. The twenty-four hour clock rhythm is closely associated with the maintenance of normal function in several endocrine systems including the triggering of the pre-ovulatory gonadotrophin surge in the female rat (Everett et al, 1977) and the ACTH/cortisol diurnal secretion (Weitzman et al, 1971; Gallagher et al, 1973). From preliminary studies in rats using simple biological parameters such as daily weight gain or other endocrine marker rhythms, Ramaley (1980) suggested that the onset of puberty may be

temporally correlated with the maturation of endogenous biological rhythms generated by the suprachiasmatic nucleus of the hypothalamus. The onset of puberty may be associated with the development of the capacity of the individual to reset endogenous biological rhythms in response to environmental cues such as the light-dark or sleep-wake cycles and to link the endogenous GnRH neuro-secretory rhythm to clock rhythms. This hypothesis has its parallel in the seasonal breeding of mammals whose sexual cycles are controlled by the interaction between the hypothalamic-pituitary-gonadal axis and another environmental variable - seasonal change in day-length or photoperiod (Licoln & Short, 1980). The entrainment of gonadotrophin or GnRH secretion to the sleep phase of the sleep-wake cycle in humans may be a further example of the biological role of synchronization of endogenous biorhythms to variables in the external or internal environments.

The concurrent acceleration of physical growth and sexual maturation during puberty has been discussed (1.2). The increase in body size and weight is associated with changes in body composition - for example increase in percentage fat per unit body weight in females and the reverse in males. The important question arises as to whether these changes in body composition or metabolism could provide biological signals outside the CNS to activate the dormant neuroendocrine mechanisms at the onset of puberty. The initial studies of Kennedy & Mitra (1963) indicating that puberty in rats occurred at a set

body size regardless of age or growth rate was used by Frisch to construct an analogous model for humans (Frisch & Revelle, 1970 & 1971). They suggested that menarche in pubertal girls was related to the attainment of a critical body weight of 47 Kg or degree of fatness - 26-28% fat per unit body weight (Frisch & McArthur, 1974). This hypothesis was supported by the association of secondary amenorrhoea and pubertal patterns of gonadotrophin secretion with anorexia nervosa (Boyar et al, 1974b). Increased body fat is associated with enhanced aromatization of androgens to oestrogens (Grodin et al, 1973) and decreased transformation of oestrogens to non-uterotrophic 2-hydroxylated metabolites, catechol-oestrogens (Fishman, 1976). This state of relative hyper-oestrogenization may stimulate the neuroendocrine mechanisms of puberty. This superficially attractive hypothesis has many flaws which have prevented its wide acceptance. The initial rat experiments on nutritional influences of sexual maturation could not be confirmed (Glass & Swerdloff, 1980). The cross-sectional data on body-fat content was calculated by indirect methods whose validity cannot be substantiated (Reeves, 1979). The postulated mechanisms cannot possibly apply to boys and such a fundamental sex difference in the earliest stages of puberty is extremely unlikely. Finally, the most severe criticism levelled against the views of Frisch and colleagues is that menarche in human puberty is a late event which has no direct bearing on the events leading to the initiation of puberty. These comments however

should not be set against the original concept that changes in body composition during physical growth may provide metabolic signals for the reactivation of the hypothalamus at the onset of puberty. Further experiments to test this hypothesis must be forthcoming.

1.6 The strategy of puberty in humans

Humans have the longest period of prepubertal growth amongst mammals. This is teleologically desirable so that ample opportunity is available to establish verbal communication and learning as well as consolidating social bonding of the family unit. This contrasts with most other animals whose period of prepubertal growth is in terms of weeks and months rather than the ten years as in humans. The existence of neural-inhibitory mechanisms which suppress the hypothalamic-pituitary unit from the end of the first year until the onset of puberty may be unique to humans or higher primates that have a prolonged period of prepubertal growth and development. It should also be noted that larger animals tend to have later puberty (Raimirez, 1973) so that attainment of an adequate physical size not only for reproduction but also for foraging and protection of offspring may be another factor in the prolonged inhibition of pubertal onset.

1.7 Rationale and approach to present studies

The hypothalamic-pituitary control of gonadal functions in the adult male is a comparatively stable system without the cyclical pattern which characterizes

the female menstrual cycle. Similarly, the dampened activities of the hypothalamic-pituitary-testicular axis in childhood are not ideal for the study of control mechanisms therein. The dramatic alterations in dynamic relationships between individual components of the hypothalamic-pituitary-testicular axis, telescoped into the pubertal years, offer a good opportunity for examining the functional characteristics of the system.

From the clinical standpoint, puberty is the time when hypogonadal disorders first become apparent. Unfortunately, due to the wide normal age range at the onset of puberty and the low levels of reproductive hormones in prepuberty, it is difficult to make an early diagnosis of hypogonadotrophic hypogonadism in contradistinction to constitutional delayed puberty. By definition, 2.5% of the population in the pubertal age group may have delayed onset of puberty (Prader, 1975). This presents a considerable clinical problem and the importance of precise hormonal definition of stages of sexual maturation cannot be overemphasized.

The established views on the mechanism of puberty based on changes in negative feedback sensitivity was founded on the concepts of closed-loop control dynamics and information gleaned from single sample cross-sectional studies of basal hormone levels. The more recent findings derived from multiple and frequent hormone estimations over prolonged periods confirmed the existence and indeed the onset of pulsatile hormone secretion and diurnal variations in the secretion rates

in pubertal subjects. These observations fundamentally altered the concepts on the mechanisms of puberty, conferring a pivotal role to the CNS modulation of pituitary and gonadal functions via hypothalamic pulsatile GnRH secretions. To date, clinical data supporting the neuroendocrine hypothesis of pubertal onset is still relatively scanty.

The present studies attempt to analyse four inter-related aspects of the hypothalamic control of pituitary and testicular functions simultaneously in a group of subjects at various stages of puberty. Firstly, the twenty-four hour periodicity of gonadotrophin and gonadal steroid secretions entrained to the sleep-wake cycle is examined in order to define its temporal relationships with other hormonal and physical events in puberty. This allows a more accurate description of the pubertal hormonal changes than previously possible and should therefore improve our understanding of the mechanisms of puberty as well as highlighting the clinical potential of nocturnal hormone-sleep studies. Secondly, the analysis of nocturnal LH pulsatile secretion permits an indirect assessment of the frequency and to some extent the amplitude of hypothalamic GnRH secretion from the earliest stages of puberty onwards. Since the pituitary responsiveness in these patients is also known, the amplitude of GnRH pulsatile secretory episodes may be evaluated. The physiological mechanism and relative importance of GnRH pulse frequency and amplitude modulation of pituitary and testicular function

in puberty may thus be clarified. Thirdly, pituitary responsiveness during puberty is assessed by a multiple low-dose GnRH stimulation regime in order to reproduce the endogenous physiological pattern. By considering the pituitary response in terms of an immediately releasable and a reserve gonadotrophin pool, it may be possible to gain some insight into the relative importance of gonadal steroid negative feedback and the priming effects of GnRH on the pituitary during pubertal development. Fourthly, the testicular response to endogenous and exogenous GnRH/LH pulsatile stimulation can be assessed and compared. This may throw some light on the importance of pulsatile trophic stimulation on Leydig cell steroidogenesis.

By evaluating how much, how frequently and when hypothalamic GnRH secretion occurs and correlating the effects of pulsatile GnRH stimulation on the pituitary and testes, it may be possible to confirm that sleep-related central mechanisms, translated into endocrine signals by the hypothalamus, are the prime movers behind the onset and progression of the final lap of sexual development in man.

CHAPTER 2

PATIENTS AND METHODS

2.1 Patients

Sixteen patients attending Endocrine Clinics in the Royal Infirmary, Western General Hospital and Royal Hospital for Sick Children, Edinburgh with retarded sexual development and/or short stature were recruited for this study with the approval of the attending physician, the family practitioner and the Reproductive Medicine Ethical Sub-Committee of the Royal Infirmary and Simpson Memorial Maternity Pavilion, Edinburgh. After careful explanation of the objectives and nature of the study, written consent was obtained from each patient and his parents. The clinical details of the sixteen patients are summarized in Table 2.1. Puberty stages were rated according to the criteria of Tanner (1962) and assessment of testicular size by comparison with Prader's orchidometer (Prader, 1966). The genitalia (G), pubic hair (PH) ratings and testicular volumes combined to give an overall score (P stages) which subdivided the pre-pubertal group into stage P1A and P1B with the latter representing those late prepubertal subjects showing some testicular enlargement but no genitalia or pubic hair development.

On entry into the study, nine patients were pre-pubertal, three were in stage P2, and two each in stage P3 and P4. The age range of the group was between 14.14

Table 2.1

Summary of clinical details, including treatment and final diagnosis in 16 prepubertal and pubertal subjects followed for up to 39 months. All growth hormone deficient patients had been receiving HGH 5 IU three times weekly for over one year before first entering this study. Androgen or gonadotrophin treatment were started after a variable period of observation (1-12 months) following the last hormone study. *The exception was subject 5, who received a 12-week course of HCG (Pregnyl) 3000 IU twice weekly after the first study. As a result, the subsequent three studies in this patient have been excluded from the analyses of group data where all results were obtained before the commencement of androgen or gonadotrophin therapy. The studies where sleep stage recordings were undertaken are denoted in parenthesis.

Subjects	Chrono-logical Age (yrs)	Skeletal Age (yrs)	Height (cm)	Weight (Kg)	PUBERTY STAGE Genital Score	Pubic Hair Score	Testes Volume (mls)	Total Score	Additional Clinical Findings	Period of Follow-Up (mths)	Number of Endocrine (Sleep Stage) Study	Treatment	Puberty Rating at last assessment	Diagnosis
1 KK	24.00	15.4	168.9	65.0	G1	PH1	1/1		Anosmia. Positive family history L undescended testis	30	(ONE)	Sustanon hCG + HMG	PH4 G3 T 3/2	Kallmann's Syndrome
2 SK	14.62	12.8	144.1	54.7	G1	PH1	1/1		HGH deficient. Encephalitis aged 5 yr. Red/green colour blindness Aneurysm R radial A.	28	ONE	HCG Testosterone oenanthate	PH5 G4 T 3/3	Combined growth hormone & gonadotrophin deficiency
3 ACh	14.14	13.5	143.0	35.4	G1	PH1	2/2	P1A	Balanced translocation 3,8 chr. 46XY. t(3:8)(q27;P122) Bilateral mixed conductive/perceptive deafness. L branchial cartilege remnant excised aged 3 yr.	24	ONE	Testosterone oenanthate	PH3 G3 T 4/3	hypogonadotrophic hypogonadism
4 RC	16.71	14.5	142.3	33.3	G1	PH1	2/2		HGH deficient	39	TWO (ONE)	HCG Testosterone oenanthate	PH4 G5 T 4/4	Growth hormone deficiency
5 ST	15.48	12.5	153.9	41.1	G1	PH1	2/2		L orchidopexy aged 15	26	*FOUR (ONE)	HCG for 12 weeks only	PH5 G5 T 12/8	Constitutional delayed puberty
6 ACal	15.90	11.5	142.5	35.7	G1	PH1	3/3		-	21	TWO (ONE)	Testosterone oenanthate total of 300 mg over 6 mth only	PH2 G3 T 12/10	Constitutional delayed puberty
7 RS	14.98	10.8	139.2	30.0	G1	PH1	3/3	P1B	-	6	ONE	-	Family doctor reported satis-factory progress 1 yr later	Constitutional delayed puberty
8 AR	14.89	11.7	137.5	48.4	G1	PH1	3/3		HGH deficient. Obesity. Bilateral cataracts. IQ 86. Bilateral orchidopexy aged 11 yr.	28	TWO	HCG Testosterone oenanthate	PH4 G4 T 4/4	Growth hormone deficiency
9 DC	15.10	13.7	132.8	29.1	G1	PH1	4/4		Emotional/social deprivation	15	(TWO)	-	PH3 G3 T 10/8	Constitutional delayed puberty
10 GG	14.73	12.8	149.0	38.9	G2	PH1	4/4		-	12	(ONE)	-	PH3 G4 T 15/12	Constitutional delayed puberty
11 CA	15.66	14.8	146.6	43.8	G2	PH1	5/5	P2	-	16	TWO	-	PH2 G3 T 10/10	Constitutional delayed puberty
12 CR	14.58	13.4	148.2	38.9	G2	PH2	5/5		-	26	FOUR (THREE)	-	PH4 G5 T 20/15	Constitutional delayed puberty
13 DF	16.58	14.5	152.3	37.3	G3	PH2	8/6	P3	-	16	(THREE)	-	PH4 G5 T 20/15	Constitutional delayed puberty
14 GFitz	15.89	13.0	145.5	44.8	G3	PH3	8/8		-	30	(THREE)	-	PH5 G5 T 15/15	Constitutional delayed puberty
15 GMcK	16.11	14.7	162.3	54.1	G4	PH3	12/12	P4	Insulin-dependent diabetes mellitus - well controlled	12	ONE	-	PH5 G5	Constitutional delayed puberty
16 GF	18.50	15.0	155.1	43.2	G4	PH4	12/12		-	30	(THREE)	-	PH5 G5 T 20/20	Constitutional delayed puberty

Table 2.1

to 24.00 years with a mean ± SD of 16.3 ± 2.67 years and median age of 15.57 years. All patients underwent full pituitary assessment with insulin hypoglycaemia, TRH and GnRH stimulation tests prior to entry into the present study. The oldest of these patients (subject 1) has Kallmann's syndrome (hypogonadotrophic hypogonadism with anosmia) and presented with a complete lack of sexual development and left undescended testis. He was included as a member of the prepubertal group. Three patients (subjects 2, 4 and 8) were growth hormone deficient and had been receiving MRC growth hormone injections, 5 I.U. three times weekly. Subjects who had already entered puberty at the start of the study were included to provide the widest possible cross-sectional spectrum of sexual maturity for testing. The study group was followed up clinically for periods varying between six to 39 months with a mean ± SD duration of 22.4 ± 8.5 months. The patients were assessed clinically at three and six-monthly intervals when height, weight, and puberty stage were recorded and testes size measured. Six patients (subjects 1,2,3,7,10 and 15) underwent sleep/hormone profile study on only one occasion at the beginning of the period of observation. Five patients (subjects 4,6,8,9 and 11) were studied on two occasions six to ten months apart. Three patients (subjects 13,14 and 16) were studied three times at six-monthly intervals. The remaining two patients (subjects 5 and 12) were studied on four occasions, the first three at six-monthly intervals and the fourth being carried out fourteen months after the third study.

With the exception of subject 5, no patient received any treatment with reproductive hormones prior to their last or only sleep/hormone profile study. Subject 5 received a twelve-week course of human chorionic gonadotrophin (Pregnyl, Organon) 1500 I.U. twice-weekly immediately following the first study. However, further pubertal maturation occurred spontaneously without continuing treatment. For this patient, the endocrine data from the second to the fourth study was therefore not included in the group analyses. In the six patients that required treatment, testosterone oenanthate (Primoteston Depot, Schering) or human chorionic gonadotrophin, hCG (Pregnyl, Organon) were commenced at various intervals after the last sleep/hormone profile study. Subject 6 received only a total of 300 mg of testosterone oenanthate over a six-month period and it was reported by his family doctor that he subsequently developed to full sexual maturity with no further treatment. Two growth-hormone deficient subjects (4 & 8) were started on testosterone even though they showed significant nocturnal LH secretion in the second study. This was because the psychosocial circumstances in these two subjects were such that further delay in virilization was undesirable (see Chapter 6).

2.2 Blood sampling technique

Blood samples were obtained at twenty minute intervals throughout the twenty-one hour period of study via an indwelling 18G Venflon intravenous cannula (Viggo AB, Helsingborg, Sweden) attached to a three-way stopcock with luer-lock connector (Vygon, Aachen, West Germany)

placed in the ante-cubital vein and kept patent between samples by 0.2-0.3 ml of heparinized saline (400 units heparin/ml of normal saline). After discarding the initial 0.5 ml of each sample, 4 or 10 mls of blood were collected in lithium-heparinized plastic tubes, centrifuged within ten minutes at room temperature at 500 G for ten minutes and the plasma separated and kept frozen at -20^{o}C until assay.

During sleep, an extension cannula (200 cm manometer line, Portex, Hythe, England) which passed through a hole in the bedroom wall to the next room was used so that blood sampling can continue without the sleeping subjects being disturbed (Fig 2.1). The length of the cannula was adequate to allow for a reasonable degree of freedom of movement during the night. To ensure that the sampling channel remained air-tight and intact during sleep, a special connecting device was made from two male luer-lock metal connectors soldered together so that it could be securely attached to the intravenous cannula at one end and the extension cannula at the other. The dead space of 5 ml in this system was primed by heparinized saline initially and cleared with the same volume at the end of each blood sampling. Removal of heparinized saline from the dead space of the sampling system and 4 or 10 ml of blood under favourable conditions and the refilling of the dead space with heparinized saline could usually be accomplished in under three minutes. Selection of the largest forearm veins for cannulation, adequate splinting of the arm at optimal positions and

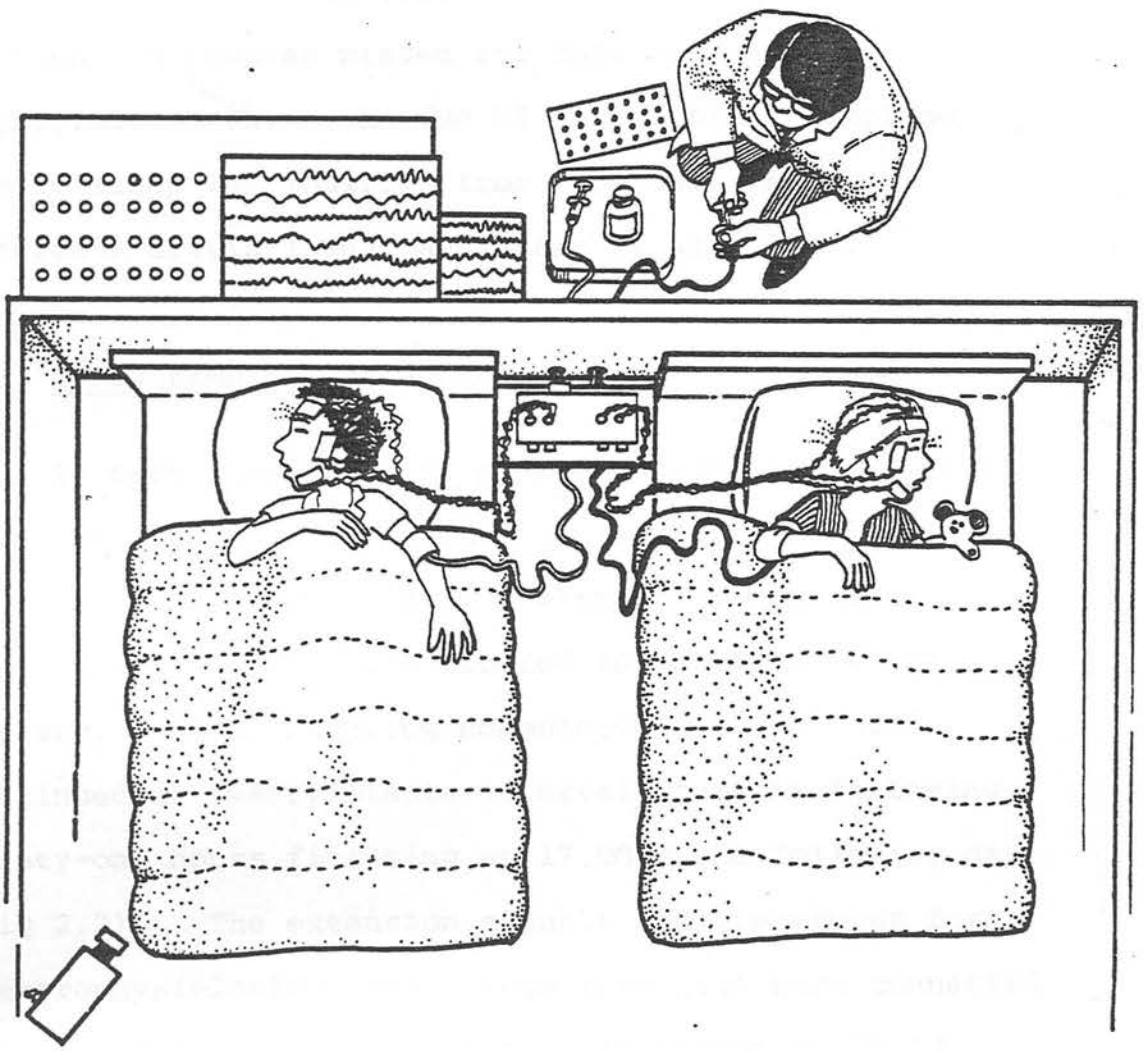


Fig 2.1
Artist's impression (courtesy of Dr T.A. Bramley) of the sleep laboratory. The subjects slept undisturbed while, in an adjacent room, blood sampling via an extension cannula running through a hole in the wall can continue through the night. The scalp, orbital and submental electrodes were connected to the electroencephalograph for recording polygraphic sleep.

the ensurance of a good flow rate through the cannula in the period before sleep were important in minimizing the potential difficulties in blood sampling during sleep. Spontaneous venous spasm, for which there was little remedy or precaution, seldom lasted for longer than 30 minutes and samples missed for this reason were negligible. The technique of serial blood sampling during sleep was modified from that described by Vankirk & Sassin (1969) and Rubin et al (1971).

2.3 Blood sampling schedule

In each sleep/hormone profile study, two patients were sampled together. They arrived in the sleep laboratory at 18.00 - 18.30 h when the intravenous cannula was inserted and secured in position in the forearm. Blood sampling commenced at 20.00 h and continued at twenty minute intervals for the following twenty-one hours finishing at 17.00 h the following day (Fig 2.2). The extension cannula and electrodes for electrophysiological recordings (see 2.4) were connected and prepared in the hour before lights-out at 23.00 h. The patients were awakened at 07.00 h the next morning when the extension cannula and electrodes were disconnected. From 09.00 h, 10 μg of GnRH (Gonadorelin, Ayerst, Andover, England) was administered as an intravenous bolus injection every two hours on four occasions. Four mls of whole blood was collected at twenty minute intervals throughout the twenty-one hours for estimation

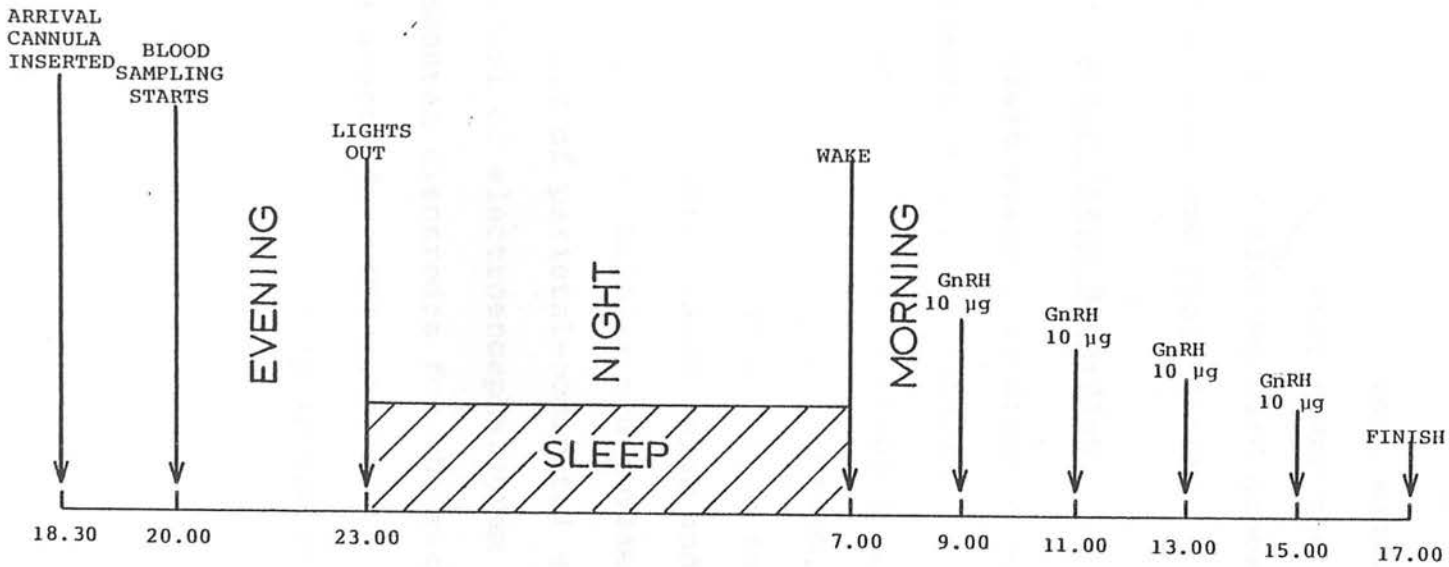


Fig 2.2

The schedule of sleep/hormone study in 16 adolescent males. After an indwelling intravenous cannula was sited 60 to 90 minutes earlier, venous blood samples were obtained at 20 minute intervals from 20.00 to 17.00 h the following day. In 10 subjects (19 studies) sleep stages were recorded polygraphically. From 09.00 h the next morning, 10 µg of GnRH (Ayerst) was administered intravenously every two hours on four occasions to assess pituitary responsiveness.

of LH, FSH and testosterone. A further 6 ml of blood was obtained at hourly intervals between 20.00 and 09.00 h for measurement of oestradiol, prolactin and for storage. The total blood volume venesected during each study was 353 ml. The patients were ambulant during the waking hours of study. They spent the time reading, watching television or playing card games and had unrestricted but normal food and fluid intake.

2.4 Sleep Stage Recording

Sleep stage recordings were performed in ten patients in a sleep laboratory on one to three occasions so that a total of nineteen studies were carried out with electrophysiological monitoring. Each patient underwent four adaptation nights before the study night. Silver electrodes were placed below and above each outer canthus providing two bipolar eye-movement recording channels, one pair of parietal-occipital electrodes providing one channel of electroencephalogram recording and one pair of submental electrodes for the recording of muscle tone. The electrodes were attached to the face with adhesive tapes and to the scalp in the midline with Colloidin. Eight foot long leads connected these electrodes to a headbox leading to a Gras polygraph recording device in the next room (Fig 2.1). Recordings of electrical potentials were made at a paper speed of 15 mm/sec giving page units of twenty seconds each. The records were scored visually in twenty-second epochs according to the criteria of Rechtschaffen & Kales (1968) without

Subject	Chronological Age yr	Height cm	Weight Kg	Puberty Stage	Total Sleep Time min	Sleep Onset Latency min	Awake min	Stages 1 & 2 min (% TST)	Stages 3 & 4 min (% TST)	NREM Sleep min (% TST)	REM Sleep min (% TST)	REM Periods
1 KK	24.00	168.9	65.0	G1 PH1	368.4	51.0	62.5	215.3(58.4)	87.2(58.4)	302.5(82.1)	65.9(17.9)	2
4 RC	16.71	142.3	33.3	G1 PH1	201.5	37.9	244.5	141.6(70.3)	59.9(29.7)	201.5(100)	0	0
5 ST	15.48	153.9	41.1	G1 PH1	373.5	5.3	38.0	187.4(52.8)	104.7(28.0)	302.1(80.8)	71.7(19.2)	2
6 ACal	15.90	142.5	35.7	G1 PH1	431.8	21.4	23.8	223.9(51.8)	119.2(27.6)	343.1(79.5)	88.7(20.5)	4
9 DC	15.10	132.8	29.1	G1 PH1	404.1	15.9	64.0	129.3(32.0)	180.2(44.6)	309.5(76.6)	84.5(23.4)	5
10 GG	14.73	149.0	38.9	G2 PH1	196.7	84.7	170.7	138.0(70.2)	52.6(26.8)	190.6(96.9)	6.0(3.1)	1
9 DC II	15.83	136.0	30.9	G2 PH2	421.9	15.2	42.9	172.4(40.8)	138.8(32.9)	311.2(73.7)	110.0(26.3)	4
12 CR	14.58	148.2	38.9	G2 PH2	469.7	31.7	38.6	218.1(46.5)	147.7(31.4)	365.8(77.9)	103.8(22.1)	4
13 DF	16.58	152.3	37.3	G3 PH2	401.0	2.0	24.0	243.0(60.6)	110.6(27.6)	353.7(88.2)	47.3(11.8)	4
14 GFitz	15.89	145.5	44.8	G3 PH3	284.3	78.0	89.7	92.0(32.3)	115.0(40.5)	207.0(72.8)	77.3(27.2)	3
12 CR II	15.58	156.8	45.2	G4 PH3	446.8	24.1	9.2	156.3(35.0)	214.2(48.0)	370.5(82.9)	76.3(17.1)	4
14 GFitz II	16.45	147.0	48.2	G4 PH4	415.7	16.2	50.0	107.5(49.9)	125.7(30.2)	333.2(80.2)	82.5(19.8)	4
13 DF II	17.10	157.1	40.5	G4 PH4	409.3	4.1	71.7	244.9(59.8)	169.5(26.8)	354.4(86.6)	54.4(13.3)	3
16 GF	18.50	155.1	43.2	G4 PH4	397.3	23.7	6.0	161.0(40.5)	135.0(34.0)	296.0(74.5)	101.6(25.5)	4
12 CR III	16.78	166.6	48.1	G5 PH4	440.9	23.2	10.8	215.9(48.9)	144.4(32.8)	360.3(81.7)	80.6(18.3)	4
16 GF II	19.03	157.6	46.7	G5 PH4	396.4	23.7	64.9	213.8(53.9)	90.7(22.9)	304.5(76.8)	92.0(23.2)	3
13 DF III	17.73	162.0	45.1	G5 PH4	367.3	8.7	85.0	215.6(58.7)	126.0(34.3)	341.6(93.0)	25.7(7.0)	3
16 GF III	19.65	159.9	48.0	G5 PH5	425.9	9.2	33.9	231.5(54.3)	118.7(27.8)	350.2(82.2)	75.6(17.8)	3
14 GFitz III	17.12	150.5	58.3	G5 PH5	452.9	12.9	11.2	217.5(48.0)	146.1(32.3)	363.5(80.3)	89.4(19.7)	5

Table 2.2 The pattern of polygraphic sleep in ten male subjects studied on one to three occasions during pubertal development. NREM = non rapid eye movement, REM = rapid eye movement, TST = total sleep time

knowledge of the identity and stage of development of the subject. The visual scores were further analysed by computer to give the absolute and relative amounts and distribution of sleep stages in each study. Hormone sampling in those studies without sleep stage monitoring was carried out in a hospital side-ward without any adaptation nights but otherwise following the same sampling schedule.

The results of the sleep stage analysis in Table 2.2 showed that polygraphic sleep in all except two patients (RC & GG) was well-organized and minimally disrupted. Sleep stage content was normal and showed no consistent pattern of change with increasing maturity either in the group as a whole or in the same subject studied repeatedly.

2.5 Analysis of episodic gonadotrophin release

The fluctuation in plasma gonadotrophin concentrations during frequent sampling at twenty minute intervals subscribed to a pulsatile pattern with an abrupt rise followed by a slower exponential decline towards the baseline. Since the clearance rate of gonadotrophins did not change acutely, the observed fluctuations in plasma concentration must reflect the pulsatile release of pituitary gonadotrophins. A gonadotrophin secretory pulse was defined by the following criteria:

1) plasma concentration of gonadotrophin in at least two consecutive samples were higher than the

previous (basal) sample, and

2) the increase from basal in at least one of the peak samples was significantly higher than might be expected on the basis of the within-assay variation - greater than twice the within-assay coefficient of variation.

For each gonadotrophin pulse, the absolute amplitude was calculated from the difference between the highest peak and the basal values.

2.6 Analysis of gonadotrophin response to multiple GnRH stimulation

The GnRH response was assessed by two criteria. The absolute maximal incremental response (Δ Max) was obtained from the difference between the highest post-stimulation value and the basal value immediately preceding the GnRH stimulation. The integrated response area (Σ area) was the cumulative value of the difference in gonadotrophin concentration between the five post-stimulation samples and the basal sample immediately preceding the GnRH injection. This was directly related to the area circumscribed by the gonadotrophin response profile (Kandeel et al, 1979).

To assess the response of the four consecutive GnRH stimuli at two-hourly intervals, the first response was compared with the mean of the second, third and fourth as well as the mean of all four responses.

2.7 Statistical analyses

The data from this mixed cross-sectional/longitudinal study was analysed by the method of unbalanced mixed model analysis of covariance (Dixon & Brown, 1979). This involves the construction of a model with separate terms for the estimation of within-patient and between-patient variabilities. In testing for trends in hormonal concentrations across the stages of puberty, the following model for value Yij on subject i at stage j was used:

$$Yij = bi + cj + eij$$

where b represented the deviation of subject i from the group mean value expected at a given stage, c represented the average level expected at the stage, and e the error term representing all unexplained deviations in the data from the additive model involving b and c. In considering the hormonal trends in individual patients studied at different stages of development longitudinally, e corresponded to the failure of all lines joining two given successive stages to be parallel while b represented some of these lines lying consistently above others at given stages of puberty and c the line joining the average hormone concentration expected at specific stages. Since the data was unbalanced (i.e. each subject was at a different set of stages), a maximum likelihood method for estimating the hormonal parameters and testing for significance was used. The programme selected was BMDP3V (Dixon & Brown, 1979). This was used to test for significant stage effects in the analysis of all the

hormonal measurements and also to test whether a linear stage effect produced as good a fit as a more general "factor" for stage. This analysis was also performed using other criteria for pubertal development such as pubic hair and testis size so that it was possible to see if they gave a better fit to the hormonal model than the total score puberty stage classification.

Kendall's rank correlation test was used to examine the relationships between the various mean and pulsatile parameters of gonadotrophin secretion and the GnRH response and linear regression lines were fitted to some of these.

CHAPTER 3

HORMONAL CORRELATES OF THE PHYSICAL CHANGES IN PUBERTY

3.1 Introduction

Puberty is characterized by a series of physical changes brought about by the functionally integrated increase in activity of the hypothalamic-pituitary-testicular (HPT) axis. The state of development of the physical characteristics of puberty can be said to reflect the functional maturity of the HPT axis and is important in the clinical assessment of the progress of sexual maturation. These physical markers of puberty also provide standard criteria against which other changes of puberty can be compared. Several investigators have developed composite classifications of male sexual development (Schonfeld, 1943; Tanner, 1962; Prader, 1966; Zachman et al, 1974). Although these studies were largely based on anthropometric surveys, endocrinological changes in puberty are likely to relate more meaningfully to the physical changes of sexual maturation than to chronological age. This is due to the wide individual variation in the age of onset and rate of progression of pubertal development (Marshall & Tanner, 1970) and the fact that the physical changes at puberty are hormonally-induced (Short, 1980). A number of studies confirmed that increasing concentrations of basal gonadotrophins and testicular steroids correlated with the clinical stages of puberty (Blizzard et al, 1972; Burr et al, 1970; Kelch

et al, 1972; Faiman & Winter, 1974; Lee et al, 1974). However, considerable discrepancy in the timing, magnitude and pattern of hormonal changes exist amongst these earlier reports based on single blood samples obtained during the day. It is now accepted that gonadotrophins are secreted predominantly during nocturnal sleep in early puberty (Boyar et al, 1972b) and that both pituitary and gonadal hormones are secreted episodically (Baker et al, 1975). Thus it is important to confirm and extend the existing data on the hormonal concentrations at different stages of puberty, taking into account the sleep-related and episodic nature of gonadotrophin and testosterone secretion. A prerequisite however is to determine which of the physical markers characterizing sexual development are most closely related to the hormonal changes and to establish a rational basis for the clinical assessment of pubertal maturation.

3.2 Results

The mean concentrations of plasma LH, FSH, testosterone and oestradiol in the evening (20.00-23.00 h), night (23.00-07.00 h) and morning (07.00-09.00 h) in thirty studies from sixteen pubertal subjects grouped into external genitalia (G) and pubic hair (PH) clinical stages are presented in Figure 3.1. Similarly the mean LH pulse frequency and amplitude during the evening (20.00-23.00 h) and night combined with morning (23.00-09.00 h) are illustrated in Figure 3.2. The night and morning periods are combined because the duration of the

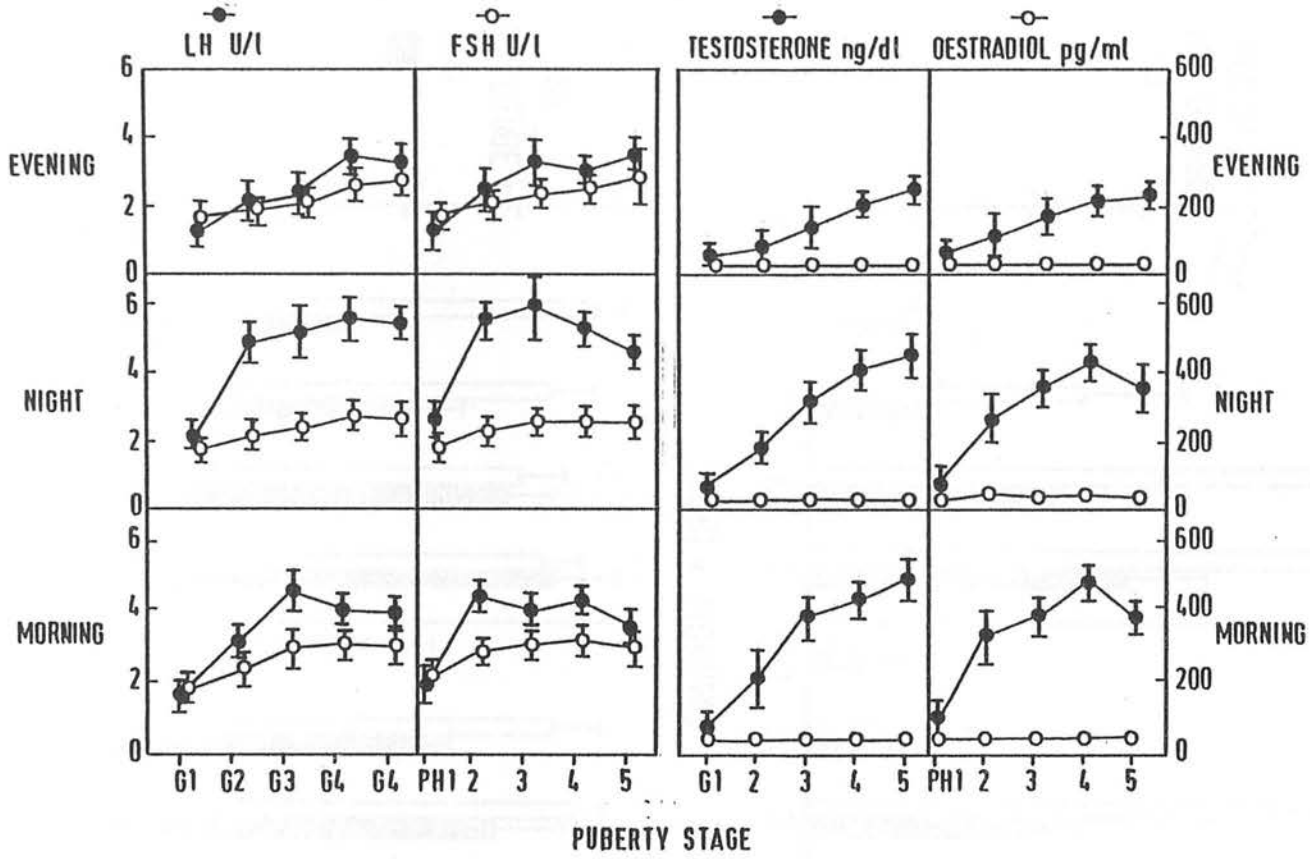


Fig 3.1 Mean (SEM) plasma LH, FSH, testosterone and oestradiol concentrations in the evening (20.00-23.00 h), night (23.00-07.00 h) and morning (07.00-09.00 h) in sixteen subjects (thirty studies) in the five stages of puberty determined either by genitalia (G) or pubic hair (PH) ratings according to Tanner (1962).

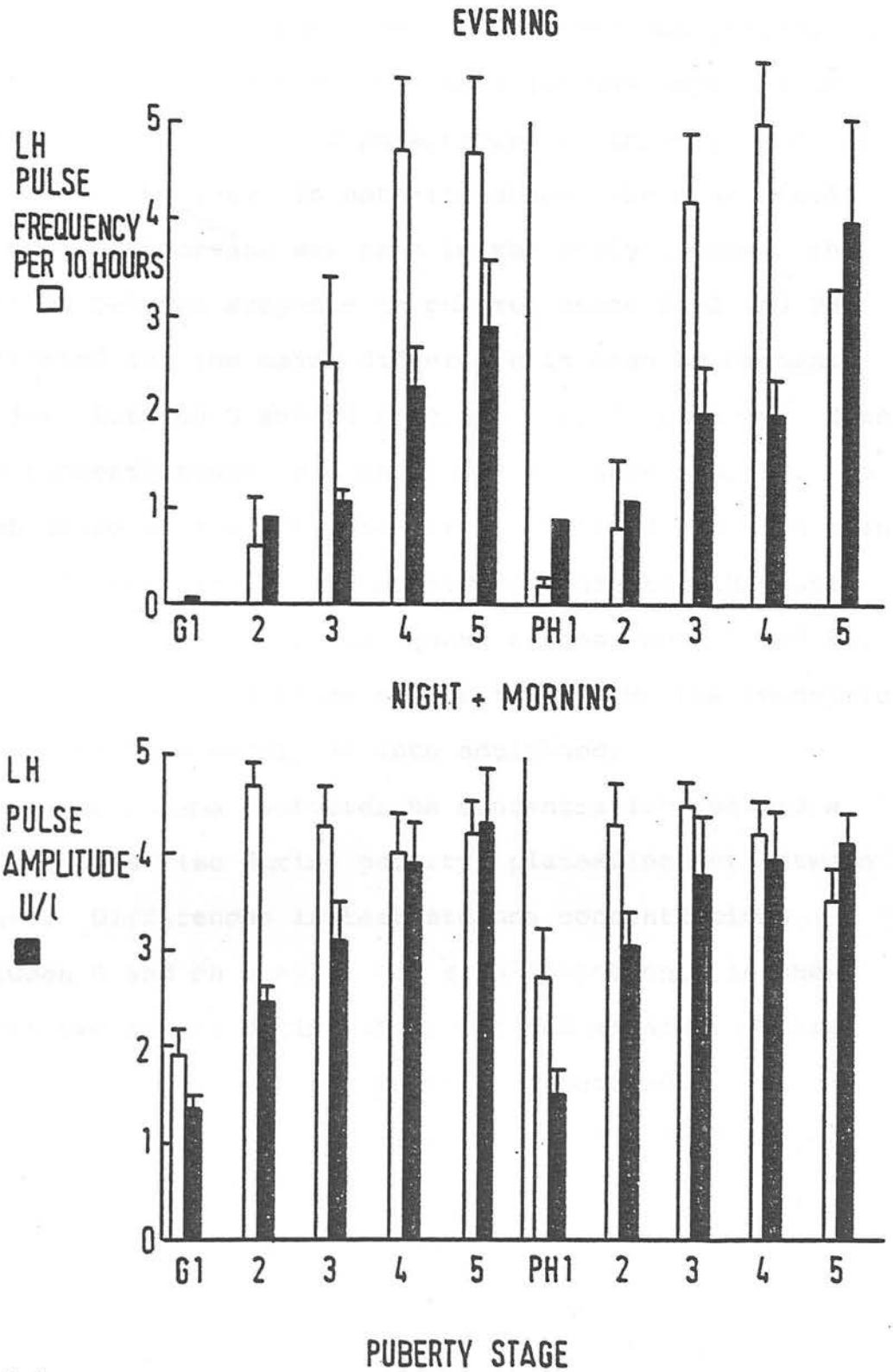


Fig 3.2.

Mean (SEM) frequency and amplitude of pulsatile LH secretion during the evening (20.00-23.00 h) and night combined with morning (23.00-09.00 h) in 16 subjects (30 studies) in the five stages of puberty determined either by genitalia (G) or pubic hair (PH) ratings according to Tanner (1962).

latter is too short to be considered on its own for pulse frequency estimations and the last LH pulses in the night were often seen to encroach into the morning period. A rising trend in mean LH concentration was apparent in both G and PH stages of puberty in all three periods of the day and night. In both instances, the most rapid rate of LH increase was seen in the early stages. The overlap between subjects in puberty stage G1-2 and PH1 accounted for the major difference in mean LH concentrations between G and PH staging in early puberty. Mean FSH concentrations in G and PH stages were similar. In both stage G1 and PH1, the ratio of LH:FSH was less than one in the evening and morning but greater than one during the night. In subsequent stages, both G and PH, LH values exceeded those of FSH throughout the twenty-four hours and this persisted into adulthood.

Mean plasma testosterone concentrations showed a progressive rise during puberty, plateauing out between PH4-5. Differences in testosterone concentrations between G and PH staging was significant only in the first two stages during the night and morning. There was no difference in the pattern of oestradiol concentrations during puberty between G and PH stages.

Significant difference in LH pulse frequency between G and PH staging was only observed in prepubertal subjects in the combined night and morning period. LH pulse amplitude showed a progressive increase throughout puberty in both G and PH stages.

The pituitary response to the first GnRH bolus and

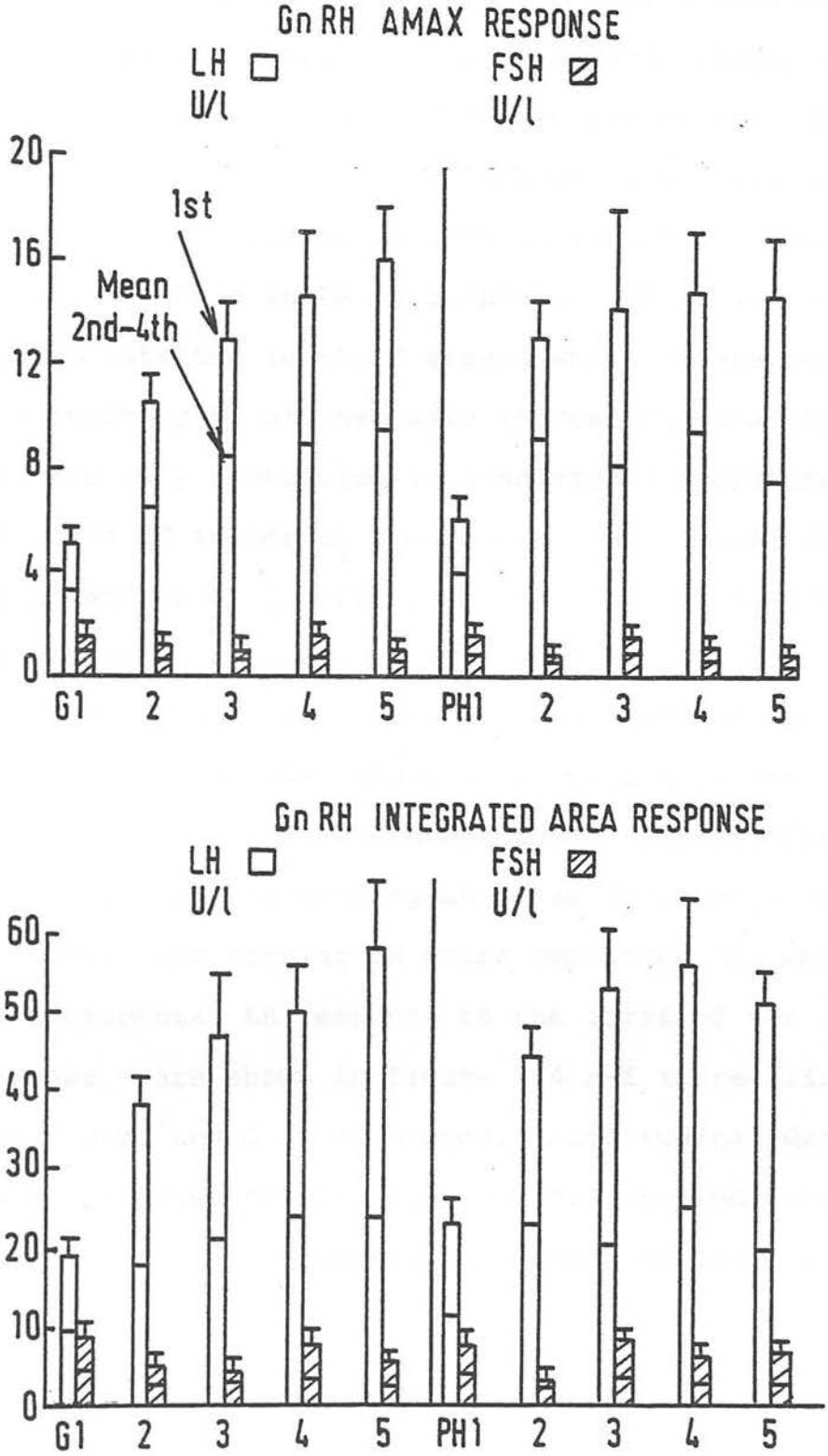


Fig 3.3 Mean (SEM) maximal incremental (Δ Max) and integrated area LH and FSH response to the first and the average of the second, third and fourth 10 μg bolus GnRH stimulation at two-hourly intervals in 30 studies on 16 subjects in the five stages of puberty determined either by genitalia (G) or pubic hair (PH) ratings according to Tanner (1962). In three studies, only one or two GnRH boluses were given instead of the usual four.

the mean response of the second to the fourth boluses assessed by maximal increment and integrated response area are shown in Figure 3.3. The two parameters of GnRH response to either the first or subsequent stimulation gave very similar patterns in both LH and FSH. The progressive increase in LH in response to GnRH was more clearly demonstrated in the G stages while PH stages showed a tendency to plateau with increasing maturity. However, the only statistically significant difference between G and PH stages in the LH response to GnRH was between G1 and PH1. The FSH response to GnRH showed no obvious change in either G or PH stages during puberty.

The individual values (N=30) of six of the most informative hormonal parameters - (a) mean nocturnal LH, (b) mean morning FSH, (c) mean nocturnal testosterone, (d) mean nocturnal and morning LH pulse frequency, (e) mean nocturnal and morning LH pulse amplitude (f) mean maximal incremental LH response to the first of the four GnRH boluses - are shown in Figure 3.4 a-f in relation to testicular size and G or PH stages. Longitudinal data from repeat studies in the same patients are indicated by straight lines joining successive hormone values. There was considerable overlap between successive G and PH stages in all five hormone parameters especially in the later stages of puberty. Testicular volume was well demarcated by G staging but showed a great deal more overlap in relation to PH stages. A linear relationship existed between the mean nocturnal LH, LH pulse frequency, LH response to GnRH and testicular volume within stages

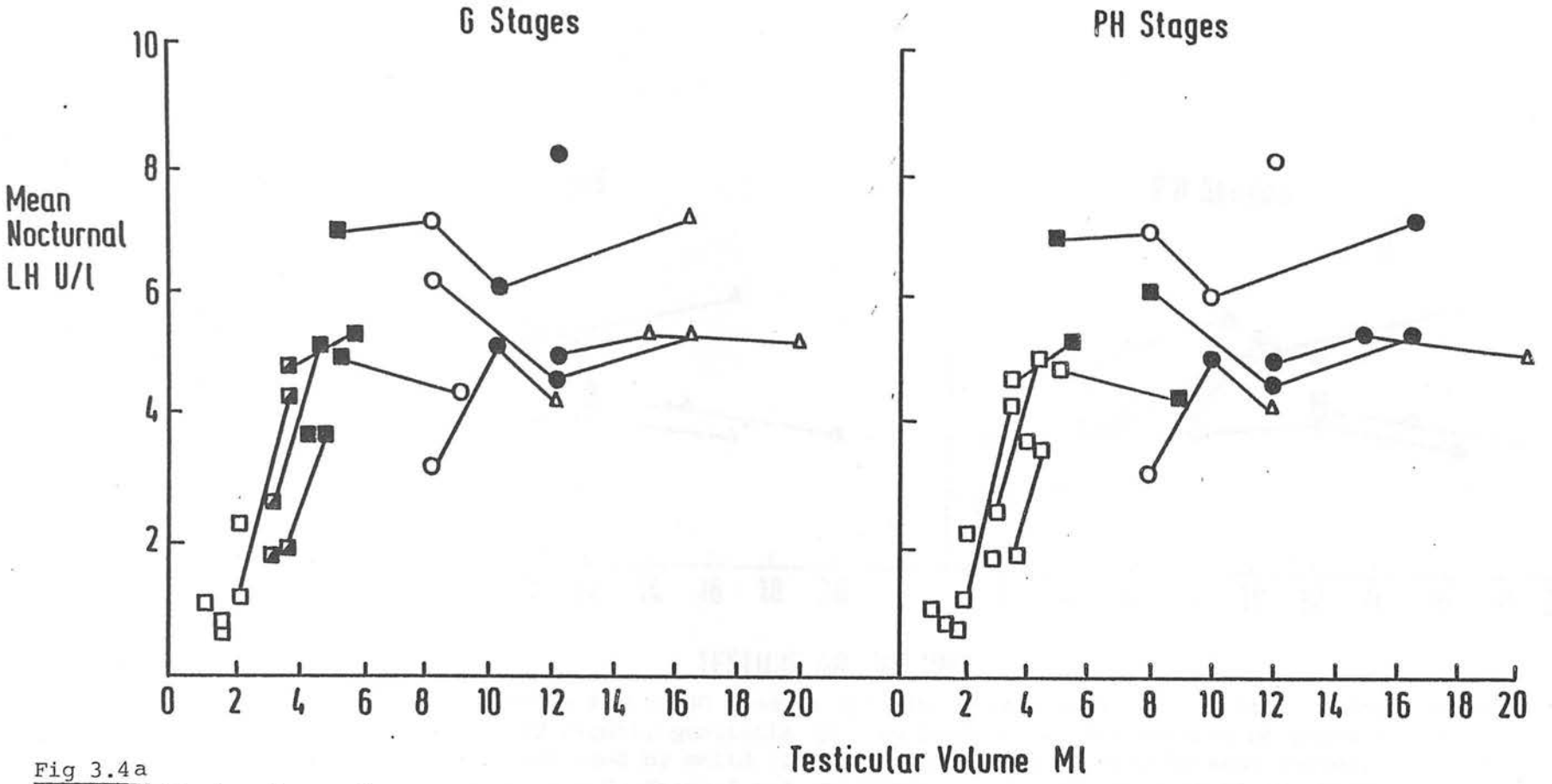


Fig 3.4a
The individual values of mean nocturnal LH in 30 studies from 16 peripubertal and pubertal subjects related to testicular volume (mean of left and right), genitalia (G) and pubic hair (PH) staging of puberty. Repeat studies in the same subject are indicated by solid lines joining the successive hormone values. G and PH stages 1 = □ , Stage 2 = ■ , Stage 3 = O, Stage 4 = ● and, Stage 5 = Δ. Patients in G1 are further divided into those with mean testicular volume ⩽2 ml = □ and >2 to ⩽4 ml = ◪ .

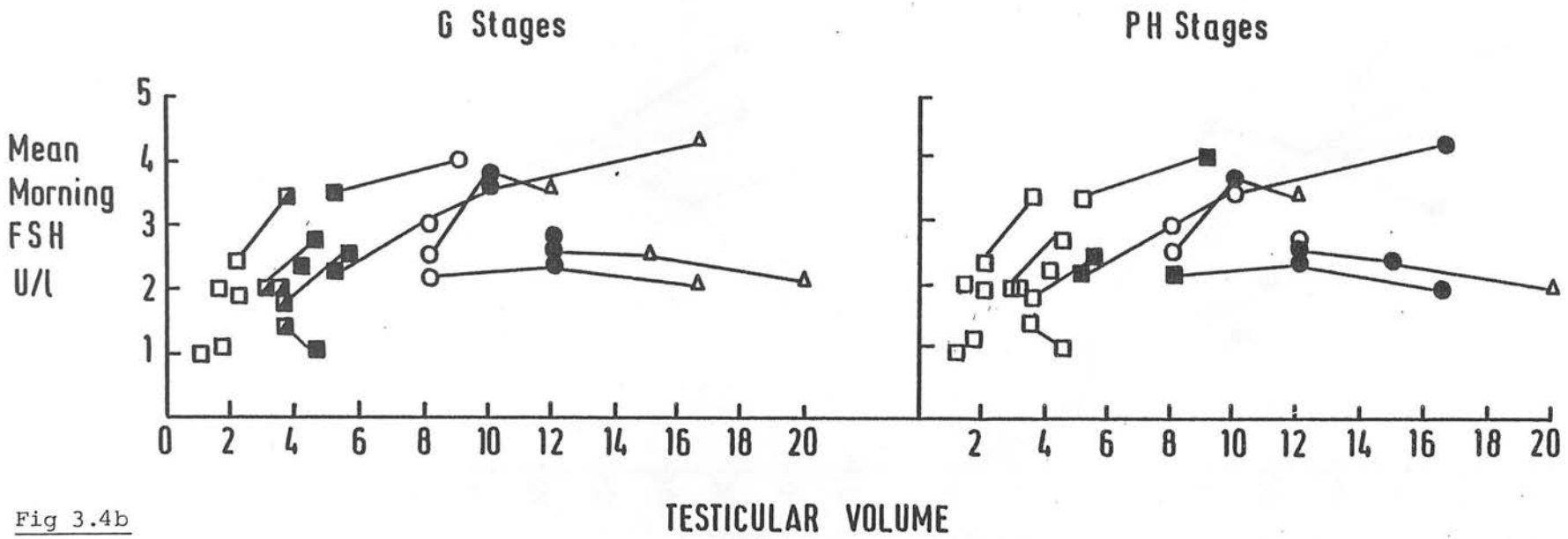


Fig 3.4b

The individual values of mean morning FSH in 30 studies from 16 peripubertal and pubertal subjects related to testicular volume (mean of left and right), genitalia (G) and pubic hair (PH) staging of puberty. Repeat studies in the same subject are indicated by solid lines joining the successive hormone values. G and PH stages 1 = □ , Stage 2 = ■ , Stage 3 = O, Stage 4 = ● and Stage 5 = Δ. Patients in G1 are further divided into those with mean testicular volume ⩽2 ml = □ and >2 to ⩽4 ml = ◪ .

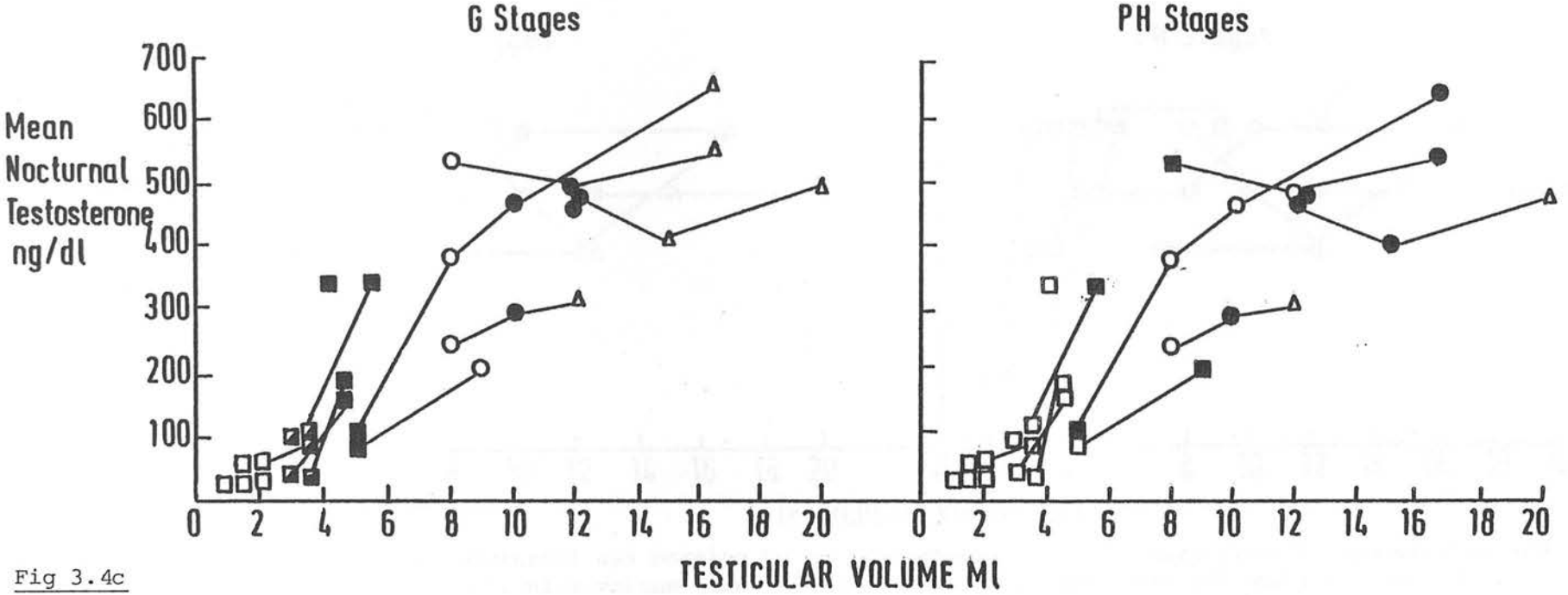


Fig 3.4c

The individual values of mean nocturnal testosterone in 30 studies from 16 peripubertal and pubertal subjects related to testicular volume (mean of left and right), genitalia (G) and pubic hair (PH) staging of puberty. Repeat studies in the same subject are indicated by solid lines joining the successive hormone values. G and PH Stages 1 = □, Stage 2 = ■ , Stage 3 = O, Stage 4 = ● and Stage 5 = Δ. Patients in G1 are further divided into those with mean testicular volume ⩽2 ml = □ and >2 to ⩽4 ml = ◪ .

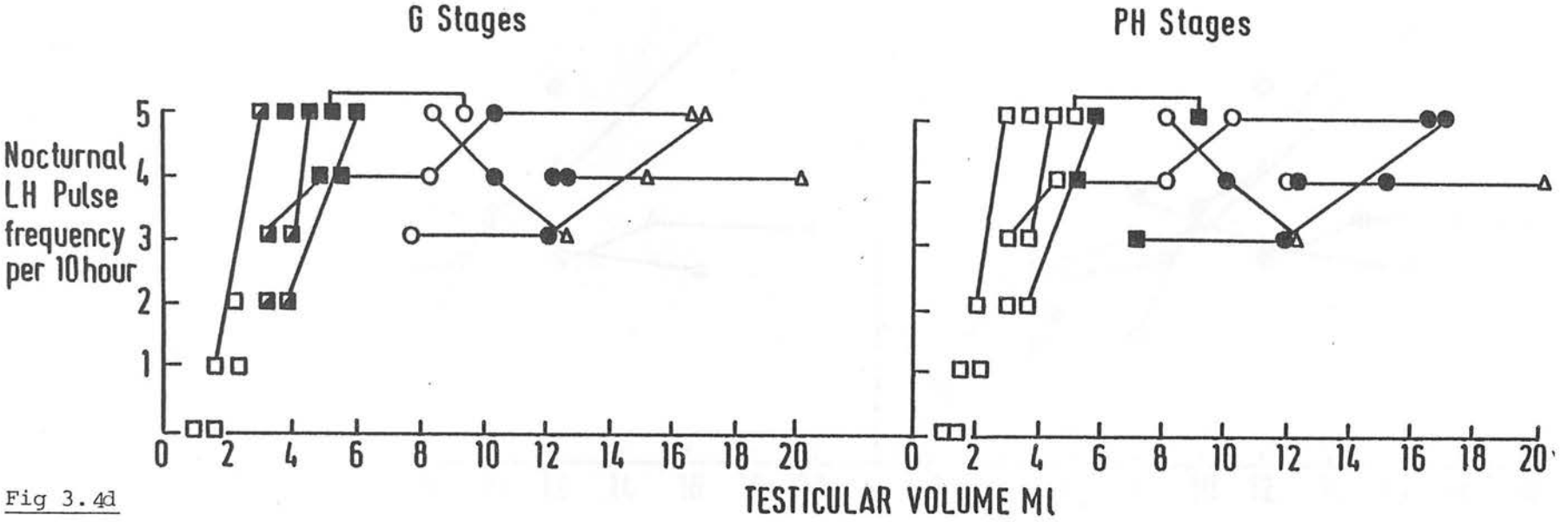


Fig 3.4d

The individual values of mean nocturnal and morning LH pulse frequency in 30 studies from 16 peripubertal and pubertal subjects related to testicular volume (mean of left and right), genitalia (G) and pubic hair (PH) staging of puberty. Repeat studies in the same subject are indicated by solid lines joining the successive hormone values. G and PH stages 1 = □ , Stage 2 = ■ , Stage 3 = O, Stage 4 = ● and Stage 5 = Δ. Patients in G1 are further divided into those with mean testicular volume ≤2 ml = ◻ and >2 to ≤4 ml = ◪.

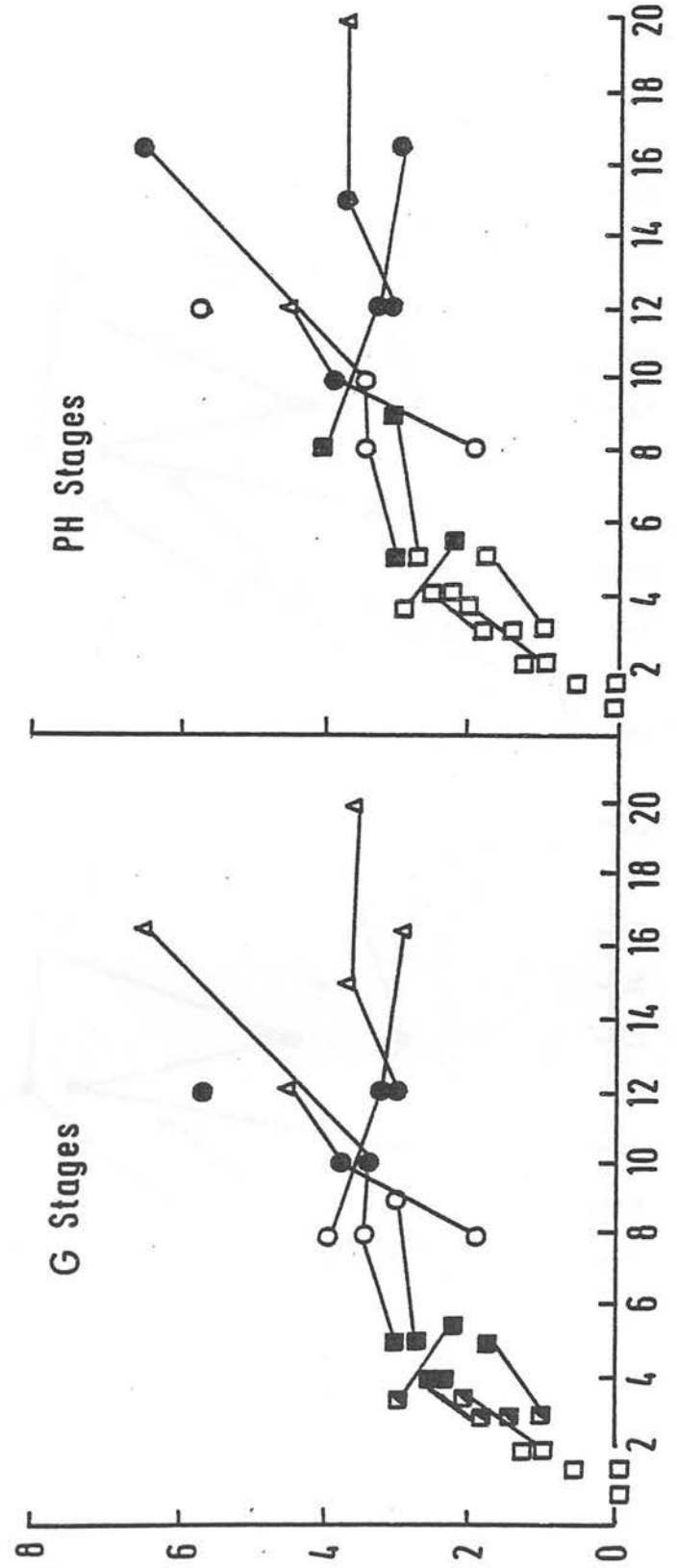


Fig 3.4e

The individual values of mean nocturnal and morning LH pulse amplitude in 30 studies from 16 peripubertal and pubertal subjects related to testicular volume (mean of left and right), genitalia (G) and pubic hair (PH) staging of puberty. Repeat studies in the same subject are indicated by solid lines joining the successive hormone values. G and PH Stages 1 = □ , Stage 2 = ■ , Stage 3 = O, Stage 4 = ● and Stage 5 = Δ. Patients in G1 are further divided into those with mean testicular volume ⩽2 ml = □ and >2 to ⩽4 ml = ◪ .

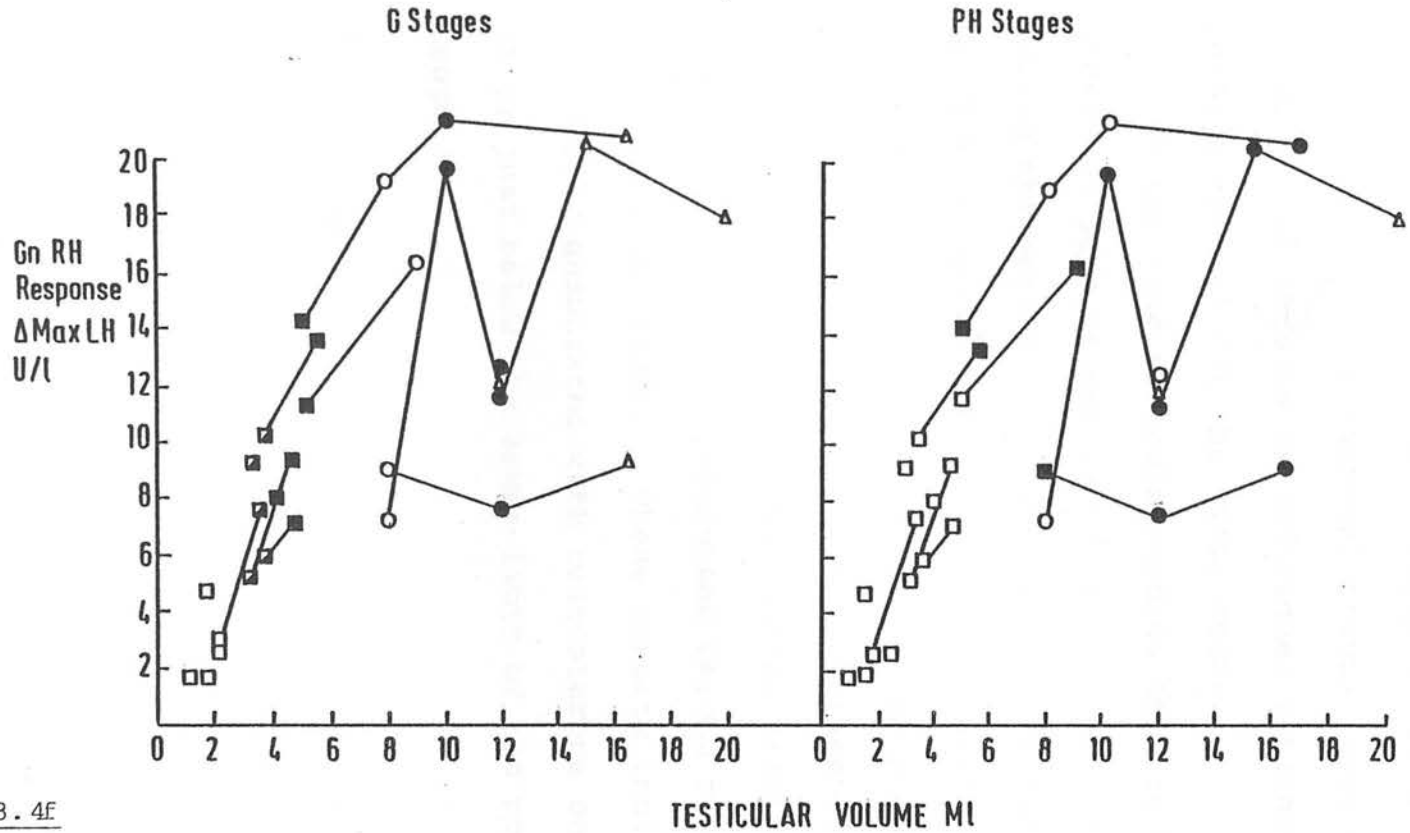


Fig 3.4f

The individual values of mean maximal incremental LH response to the first of the four 10 µg GnRH bolus stimulation in 30 studies from 16 peripubertal and pubertal subjects related to testicular volume (mean of left and right), genitalia (G) and pubic hair (PH) staging of puberty. Repeat studies in the same subject are indicated by solid lines joining the successive hormone values. G and PH Stages 1 = □ , Stage 2 = ■ , Stage 3 = O, Stage 4 = ● and Stage 5 = Δ. Patients in G1 are further divided into those with mean testicular volume ⩽2 ml = ◻ and >2 and ⩽4 ml = ◪ .

G1-2. The nocturnal LH pulse amplitude and testosterone concentration correlated with testicular volume throughout puberty. Thus the combination of testicular size and G stages provided the best clinical assessment of pubertal development in terms of their hormonal relationships. The hormonal trends revealed by the longitudinal studies in individual patients mostly paralleled that for the group means.

Of the nine subjects studied on more than one occasion, peak height velocity (PHV) was achieved in one during the period of study (subject 12 who was studied on four occasions). The PHV in this patient occurred between stage G3-4 and testes volumes between 8-10 ml with testosterone concentrations between 400-500 ng/dl. The other four patients who reached stage G5 during the study period had all progressed beyond their PHV before entry into the study. These results indicate that PHV is usually associated with testosterone concentrations at or just below the lower limit of the normal adult range.

3.3 Discussion

Since neither the chronological nor bone age are reliable indicators of pubertal maturation, determination of the precise temporal and quantitative relationships between hormonal changes in puberty must be interpreted with reference to the state of maturity evidenced by the development of sex-hormone dependent physical characteristics. To gain insight into the physiological mechanisms

of puberty, it is therefore necessary first to ascertain which of the different physical markers of puberty are most closely associated with the measurable changes in hormone secretion.

The present results confirmed that the principal difference between G and PH staging of puberty can be explained by the earlier development of genitalia changes than pubic hair growth (Marshall & Tanner, 1974; Lee et al, 1974). Thus the hormonal changes in relation to G and PH stages are likewise shifted temporally by these arbitrary classifications. The discrepancy between the two classifications was most obvious amongst subjects around the onset of puberty (Fig 3.4). This implies that pubic hair changes are further removed in time from the hormonal mechanisms responsible for the initiation of puberty and are consequently less reliable as clinical indices of early pubertal development.

Our results also confirmed that testicular enlargement (Schonfeld, 1943; Tanner, 1962; Zachmann, 1974) and increase in plasma concentration of LH and/or FSH (Burr et al, 1970; August et al, 1972; Winter & Faiman, 1972a & 1974; Lee et al, 1974; Baker et al, 1976) occurred before signs of genitalia development, appearance of pubic hair or any appreciable increase in circulating testosterone concentration. Estimation of gonadotrophin concentrations during nocturnal sleep in this study showed that in peripubertal boys, enlargement of the testes above 1-2 ml in volume was closely associated with the onset of nocturnal LH secretion (Fig 3.4a & d) which was also reflected by

the greater nocturnal LH:FSH ratio compared to the morning and evening in stage G1 (Fig 3.1). A concurrent increase in pituitary responsiveness to GnRH can also be detected at this stage (Fig 3.4f). The attainment of testicular volume of 4 ml or more indicated well established nocturnal LH secretion which was usually followed by increased testosterone secretion and further pubertal development. These considerations in the peri-pubertal period together with the finding that testicular volumes could be conveniently demarcated by subsequent G stages suggested that the combination of G staging and testicular size estimation provided the best physical criteria for the assessment of puberty. The application of this combination as the working "total" score of puberty staging (P stages) would therefore only involve modifying the established G staging system by subdividing G1 into P1A where testicular size was 1-2 ml and P1B for those with testes volumes above 2 ml but not more than 4 ml while stages P2-5 would be equivalent to G2-5.

Testicular enlargement during sexual maturation is the result of increases in seminiferous tubular diameter and length (Charny et al, 1952; Richardson & Short, 1978) and is associated with the initiation and establishment of mature spermatogenesis. Previous studies have demonstrated correlations between testicular size and circulating FSH concentration (Winter & Faiman, 1972a; Burr et al, 1970) and testosterone concentration (Knorr et al, 1974) in pubertal subjects. This study found that the increase in testicular size was only correlated with

rising LH and FSH secretion in early puberty, P1-2 (Fig 3.4 a & b). The subsequent increase in testicular size was associated with only insignificant changes in mean gonadotrophin levels. In contrast, a linear relationship between testicular size and testosterone concentration and LH pulse amplitude was maintained from stage P1A-5. These results are compatible with the hypothesis that testosterone as well as gonadotrophins are required for the initiation of spermatogenesis while the maintenance and further development of the seminiferous tubules to adult proportions is dependent on increasing concentrations of intratesticular testosterone (Steinberger, 1971; Steinberger et al, 1978). The discovery of sperms in the urine of early pubertal boys (Richardson & Short, 1978) indicating the establishment of spermatogenesis before the full development of secondary sexual characteristics is also compatible with the present findings and interpretations.

Gonadal steroids are responsible for the development of secondary sexual characteristics as well as the more general metabolic changes in puberty (Short, 1980). Testosterone rather than dihydrotestosterone is the major effector hormone in male puberty since subjects with 5 - reductase deficiency can go through a virtually normal puberty and also initiate qualitatively normal spermatogenesis (Peterson et al, 1977). Unbound circulating testosterone increases in puberty to a greater extent than total testosterone due to a fall in sex hormone binding globulin (SHBG) concentration (August et al,

1969). However, there may be some delay in this fall in SHBG after the increase in total circulating testosterone concentration in puberty (Lee & Gisriel, 1980). This may, to some extent, explain the considerable latent period between androgen target tissue response observed clinically and the increase in total plasma testosterone concentration. It does not, however, account for the temporal dissociation between pubic hair and genitalia development, which must be explained at the level of target cell responsiveness. These factors affecting circulating testosterone presumably do not apply to the seminiferous tubules, being adjacent to the source of androgen synthesis where androgen can diffuse freely across the blood-testis barrier (Setchell, 1980). This ensures that seminiferous tubules are the first tissue to be exposed to sufficient concentrations of testosterone from the earliest stages of sexual maturation. Furthermore, yet another safety device exists in some species in the form of an intratubular androphilic macromolecule - androgen binding protein (Fritz, 1978) which may prevent the rapid metabolism of testosterone and further enhance its local availability within the seminiferous tubules. These differences in proximity and transport mechanisms for testosterone may account for the early response to androgens of seminiferous tubules compared to the other distant androgen target organs.

In summary, pubertal development indicated by the physical changes in external genitalia, pubic hair and testicular size were compared in terms of their

relationships with hormonal changes represented by the nocturnal episodic secretion of gonadotrophins and testicular steroids as well as the pituitary response to GnRH stimulation. Testicular size was found to be the best single physical index of sexual maturation and reflected the endocrine events in puberty more accurately than either genitalia or pubic hair development especially in the early stages of puberty. The combination of genitalia stages and testicular volume assessment provided the most satisfactory overall staging criteria for the clinical assessment of male puberty.

CHAPTER 4

HYPOTHALAMIC-PITUITARY DYNAMICS IN PUBERTY

4.1 Introduction

It is now generally accepted that puberty in animals is initiated by functional alterations in the hypothalamic-pituitary-gonadal axis. In humans, episodic gonadotrophin secretion becomes detectable in the peripheral circulation around the onset of puberty, at first only during sleep (Boyar et al, 1972b). The pituitary becomes increasingly responsive to GnRH stimulation (Kastin et al, 1972; Job et al, 1972; Roth et al, 1972) while testicular steroidogenesis is greatly enhanced in response to the rising gonadotrophin levels (Winter et al, 1972; Schöller et al, 1975). The increase in sex steroid and gonadotrophin secretion initiates spermatogenesis and development of secondary sexual characteristics (Steinberger, 1971; Tanner, 1974). Although these events are individually well-described, the timing of onset, the sequence as well as their relationships to each other have not been precisely defined.

A growing body of evidence has accumulated from clinical and experimental studies to suggest that the initiation of puberty is dependent on neuroendocrine mechanisms (Donovan & Van der Werff Ten Bosch, 1965; Critchlow & Bar Sela, 1966; Gorski, 1974) where the hypothalamus provides the crucial link between the neural

and hormonal components (Grumbach, 1980; Knobil, 1981; Desjardins, 1980). The neural signal is translated into the frequency-coded pulsatile GnRH secretion of the hypothalamus, the activation of which is central to the awakening of the pituitary-gonadal axis at the onset of puberty (Licoln & Short, 1980; Goodman & Karsch, 1981). Since GnRH is found in the peripheral circulation in quantities too low to be reliably measured by conventional RIA (Kelch et al, 1975; Mortimer et al, 1976), episodic changes in circulating gonadotrophin levels have come to be regarded as an indirect but valid index of pulsatile GnRH secretion by the hypothalamus (Carmel et al, 1976; Eskay et al, 1977; Neill et al, 1977; Licoln & Fraser, 1979). The transient entrainment of pulsatile gonadotrophin secretion to sleep at the onset of puberty may be a manifestation of an underlying neuroendocrine mechanism important in the initiation of human puberty (Boyar, 1978). As yet, the physiological significance of this overt neuroendocrine phenomenon has not been studied in relation to the other well-described changes in functional dynamics in the hypothalamic-pituitary-testicular axis in pubertal subjects. By using frequent blood-sampling techniques during nocturnal sleep as well as during the day this study aims to analyse the endogenous GnRH/ gonadotrophin secretory patterns and diurnal profiles of gonadotrophin and sex steroid secretion at specific stages of puberty. Concurrently, the pituitary and testicular responsiveness to multiple submaximal doses of endogenous GnRH is assessed in a manner similar to the

endogenous pulsatile stimulation. By collating these data in a mixed longitudinal and cross-sectional group of pubertal subjects at various stages of maturity, it may be possible to gain further insight into the mechanisms underlying the initiation and progression of pubertal development in the human male.

4.2 Results

Individual profiles of plasma LH, FSH and testosterone in the sixteen patients studied on one to four occasions are shown in Appendix II. The mean basal hormone concentrations, the GnRH response, and the nocturnal LH pulse frequency and amplitude in the thirty studies from sixteen subjects are shown in increasing order of maturity in Table 4.1. The same data grouped into the six clinical stages of puberty are shown in Tables 4.2a, 4.2b and 4.2c.

4.2.1 Hormone profiles

Representative hormone profiles from three different subjects in pre, early and late puberty are depicted in Figures 4.1a, 4.1b and 4.1c respectively. Spontaneous LH but not FSH pulsatile secretion was observed in each of the three phases of puberty although the characteristic LH pulse frequencies and amplitudes are quite different. A similar pattern of change in LH pulsatile secretion from pre to late puberty could also be observed in the longitudinal series

Table 4.1

The mean (SD) LH, FSH, testosterone, oestradiol and prolactin; LH pulse frequency and amplitude; the LH and FSH (maximal incremental and integrated response area) response to four 10 μg GnRH bolus stimulation and the mean testosterone concentration during GnRH stimulation in 30 individual studies from 16 pubertal subjects. Repeat studies in 9 subjects are indicated by roman numerals. Evening (E) 20.00-23.00 h, night (N) 23.00-07.00 h and morning (M) 07.00-09.00 h. Each basal hormone value represents the mean of 10, 24 and 6 samples respectively taken at 20 minute intervals during these three periods.

SUBJECT	Puberty Stage	Testes Vol.	LH u/l Mean (SD)			FSH u/l Mean (SD)			Testosterone ng/dl Mean (SD)			Oestradiol pg/ml Mean (SD)			Prolactin mu/l Mean (SD)			LH Pulse Evening		LH Pulse Night and Morning	
			E	N	M	E	N	M	E	N	M	E	N	M	E	N	M	Freq/ 10h	Amp u/l	Freq/ 10h	Amp u/l
1	G1	PH1 1/1	0.77 (0.09)	0.73 (0.09)	0.80 (0.06)	1.01 (0.09)	0.97 (0.09)	1.03 (0.05)	42.7 (8.6)	40.6 (11)	53.5 (5.6)	37.0 (8.6)	23.4 (4.0)	25.5 (4.5)	179 (17)	330 (74)	207 (39)	0	0	0	0
2	G1	PH1 1/1	0.86 (0.05)	0.96 (0.11)	0.95 (0.08)	0.86 (0.05)	0.79 (0.05)	0.90 (0.06)	26.0 (3.9)	18.7 (4.7)	19.8 (1.2)	21.5 (0.6)	26.8 (3.1)	29.0 (8.0)	882 (891)	484 (240)	320 (81)	0	0	0	0
3	G1	PH1 2/2	0.52 (0.04)	0.71 (0.15)	0.52 (0.04)	1.78 (0.17)	1.85 (0.13)	1.88 (0.13)	31.7 (8.9)	36.7 (14)	38.8 (13)	12.0 (3.0)	12.4 (1.4)	13.0 (0)	-	-	-	0	0	1	0.5
4	G1	PH1 2/2	1.05 (0.15)	1.06 (0.26)	0.88 (0.04)	2.39 (0.23)	2.26 (0.26)	2.33 (0.09)	57.0 (5.4)	49.8 (8.7)	60.3 (8.4)	19.0 (1.4)	20.0 (3.3)	18.0 (0)	149 (17)	408 (155)	249 (32)	0	0	1	1.0
5	G1	PH1 2/2	1.17 (0.17)	2.17 (0.35)	1.35 (0.10)	1.46 (0.08)	1.81 (0.26)	1.78 (0.07)	20.0 (0)	28.0 (9.2)	20.0 (0)	22.0 (3.6)	20.7 (2.0)	24.0 (0)	88 (20)	426 (149)	158 (48)	0	0	2	1.15 (0.15)
6	G1	PH1 3/3	0.67 (0.08)	1.81 (0.60)	1.28 (0.31)	0.97 (0.07)	1.14 (0.17)	1.35 (0.10)	12.4 (3.2)	23.9 (11)	35.0 (12)	29.0 (3.9)	24.0 (2.2)	21.0 (3.0)	800 (572)	981 (567)	690 (843)	0	0	3	0.93 (0.12)
7	G1	PH1 3/3	0.87 (0.11)	1.76 (0.89)	1.97 (0.39)	1.78 (0.10)	1.69 (0.20)	1.92 (0.19)	38.1 (7.7)	84.9 (45)	164.5 (14)	-	-	-	-	-	-	0	0	2	1.45 (0.05)
8	G1	PH1 3/3	1.05 (0.10)	2.50 (0.80)	1.37 (0.09)	1.65 (0.14)	1.79 (0.19)	1.88 (0.09)	21.0 (3.8)	33.0 (9.5)	31.3 (3.3)	19.3 (1.9)	17.8 (4.0)	18.0 (0)	178 (47)	363 (173)	152 (29)	0	0	3	1.77 (0.45)
4 II	G1	PH1 3/4	2.25 (0.21)	4.26 (1.27)	3.43 (0.86)	2.56 (0.20)	2.56 (0.38)	3.35 (0.15)	70.8 (6.8)	81.7 (14)	97.0 (12)	18.5 (1.0)	22.1 (5.5)	24.5 (2.5)	326 (66)	499 (132)	412 (147)	0	0	5	2.04 (0.63)
9	G1	PH1 4/3	2.11 (0.25)	4.71 (1.01)	2.37 (0.24)	1.57 (0.08)	1.74 (0.16)	1.72 (0.07)	44.7 (4.0)	101 (32)	83.0 (12)	23.5 (4.1)	27.0 (3.5)	30.0 (0)	463 (103)	478 (109)	178 (38)	0	0	2	2.90 (0.40)
8 II	G2	PH1 4/4	3.17 (0.39)	5.10 (1.40)	4.03 (0.51)	2.30 (0.10)	2.30 (0.30)	2.70 (0.25)	74.4 (9.7)	156 (30)	137 (14)	17.0 (2.9)	17.9 (3.8)	25.0 (0)	244 (42)	458 (223)	335 (98)	0	0	4	2.28 (0.26)
10	G2	PH1 4/4	1.16 (0.11)	3.66 (1.61)	2.88 (0.77)	1.76 (0.12)	1.95 (0.23)	2.27 (0.16)	53.2 (10)	337 (136)	349 (76)	31.8 (2.9)	33.5 (2.9)	34.0 (0)	120 (10)	299 (130)	136 (24)	0	0	5	2.56 (0.85)
6 II	G2	PH1 5/5	1.31 (0.44)	3.62 (0.97)	1.72 (0.40)	0.96 (0.19)	1.12 (0.32)	1.12 (0.07)	45.5 (16)	181 (64)	162 (36)	-	-	-	239 (89)	392 (159)	228 (130)	1	0.9	5	1.76 (0.41)
11	G2	PH1 5/5	1.34 (0.64)	4.90 (1.50)	2.42 (0.60)	2.36 (0.13)	3.27 (0.36)	3.37 (0.15)	17.5 (4.0)	80.5 (29)	79.0 (8.4)	19.5 (3.3)	35.6 (5.5)	31.0 (3.0)	197 (38)	718 (590)	260 (73)	0	0	5	2.78 (0.74)
12	G2	PH2 5/5	2.06 (0.36)	6.99 (1.66)	3.18 (0.57)	1.75 (0.13)	2.03 (0.17)	2.23 (0.07)	57.2 (17)	99.9 (31)	121 (9.7)	16.3 (3.1)	22.9 (5.5)	18.0 (0)	235 (44)	458 (167)	215 (66)	0	0	4	3.00 (0.38)
9 II	G2	PH2 6/5	3.61 (0.51)	5.27 (1.33)	4.27 (0.81)	2.32 (0.37)	2.29 (0.25)	2.47 (0.07)	164 (18)	332 (128)	393 (62)	25.5 (4.4)	50.0 (7.2)	47.5 (4.5)	453 (133)	673 (152)	470 (174)	0	0	5	2.20 (0.79)
11 II	G3	PH2	1.00 (0.12)	4.33 (1.70)	5.25 (1.45)	2.49 (0.48)	3.00 (0.43)	3.97 (0.30)	31.6 (17)	201 (113)	315 (45)	-	-	-	190 (42)	315 (179)	233 (165)	0	0	5	3.02 (1.39)
13	G3	PH2 8/8	3.46 (0.60)	6.11 (1.79)	4.73 (0.70)	1.89 (0.42)	1.87 (0.15)	2.18 (0.20)	308 (70)	523 (89)	546 (17)	34.7 (2.1)	33.1 (4.3)	38.0 (0)	136 (9.9)	327 (106)	230 (94)	1	1.1	3	4.00 (1.26)
12 II	G3	PH3 8/8	2.36 (0.71)	7.12 (1.83)	5.12 (1.30)	2.01 (0.17)	2.43 (0.31)	2.95 (0.10)	85.2 (31)	374 (96)	404 (76)	18.3 (2.5)	22.5 (4.7)	24.5 (0.5)	280 (44)	467 (154)	392 (85)	1	0.9	4	3.48 (1.51)
14	G3	PH3 8/8	2.78 (0.53)	3.15 (1.03)	3.38 (0.92)	2.20 (0.16)	2.12 (0.26)	2.48 (0.23)	216 (20)	241 (46)	293 (51)	32.3 (3.2)	30.9 (4.5)	32.0 (0)	115 (9.5)	220 (165)	466 (235)	1	1.2	5	1.92 (0.94)
15	G4	PH3 12/12	3.64 (0.95)	8.21 (2.50)	3.00 (0.35)	2.29 (0.19)	2.67 (0.26)	2.73 (0.17)	196 (64)	484 (117)	422 (89)	17.0 (1.0)	23.0 (2.8)	21.5 (0.5)	-	-	-	1	3.0	4	5.78 (1.20)
12 III	G4	PH3 10/10	4.89 (1.31)	6.05 (1.90)	4.13 (0.79)	3.13 (0.28)	3.22 (0.30)	3.60 (0.12)	300 (73)	465 (68)	497 (30)	23.0 (3.7)	22.8 (5.0)	-	342 (100)	1116 (594)	641 (230)	2	2.85 (0.35)	5	3.46 (0.63)
13 II	G4	PH4 12/12	2.76 (0.51)	4.57 (0.93)	4.53 (0.65)	2.18 (1.40)	2.13 (0.19)	2.47 (0.27)	223 (50)	464 (152)	494 (81)	25.7 (4.0)	27.0 (4.0)	29.0 (2.0)	341 (52)	568 (164)	408 (111)	1	1.30	3	3.27 (0.53)
14 II	G4	PH4 10/10	3.31 (1.12)	5.03 (1.73)	3.80 (0.70)	3.19 (0.19)	3.26 (0.32)	3.72 (0.11)	196 (31)	284 (57)	323 (26)	20.3 (1.5)	25.9 (4.2)	27.0 (0)	264 (33)	495 (200)	435 (151)	1	3.20	4	3.85 (1.48)
16	G4	PH4 12/12	3.01 (0.43)	4.93 (1.55)	4.38 (0.73)	2.47 (0.19)	2.44 (0.30)	2.60 (0.24)	211 (29)	476 (63)	523 (64)	35.0 (7.5)	33.8 (4.5)	21.0 (0)	102 (6.0)	346 (186)	235 (122)	2	1.00 (0.2)	4	3.20 (1.28)
13 III	G5	PH4 18/15	2.78 (0.43)	5.41 (1.35)	3.85 (0.91)	1.73 (0.09)	1.82 (0.20)	2.05 (0.11)	2.44 (8.4)	552 (128)	618 (32)	258 (4.0)	34.1 (3.2)	34.0 (6.0)	339 (45)	702 (487)	386 (154)	2	1.15 (0.05)	5	2.98 (1.52)
12 IV	G5	PH4 18/15	2.45 (1.28)	7.25 (2.75)	3.90 (0.79)	3.44 (0.30)	3.87 (0.33)	4.32 (0.16)	337 (134)	647 (59)	632 (43)	21.5 (3.3)	23.5 (5.1)	24.0 (1.0)	260 (51)	411 (110)	255 (66)	1	0.9	5	6.60 (2.50)
14 III	G5	PH5 12/12	4.08 (1.78)	4.37 (1.61)	2.85 (0.53)	3.68 (0.35)	3.23 (0.35)	3.55 (0.11)	287 (34)	304 (44)	346 (20)	29.5 (5.8)	29.4 (4.0)	32.0 (2.0)	242 (74)	458 (245)	386 (197)	1	5.6	3	4.50 (0.80)
16 II	G5	PH4 15/15	4.11 (0.88)	5.38 (1.93)	5.22 (1.06)	2.46 (0.15)	2.21 (0.18)	2.48 (0.18)	248 (52)	402 (121)	477 (69)	25.7 (3.2)	27.4 (4.2)	26.0 (1.0)	285 (46)	1416 (783)	770 (69)	1	2.4	4	3.75 (1.20)
16 III	G5	PH5 20/20	3.12 (0.77)	5.20 (1.69)	3.93 (0.55)	2.03 (0.13)	1.97 (0.16)	2.17 (0.07)	242 (33)	485 (115)	479 (33)	23.3 (1.5)	22.9 (6.0)	30.5 (9.5)	182 (20)	1014 (626)	562 (332)	1	2.4	4	3.70 (1.47)

SUBJECT	Puberty Stage	Testes Vol.	LH Response to GnRH: Δ Max 1st	2nd	3rd	4th	Mean (SD)	LH Response to GnRH: Integrated Area 1st	2nd	3rd	4th	Mean (SD)	FSH Response to GnRH: Δ Max 1st	2nd	3rd	4th	Mean (SD)	FSH Response to GnRH: Integrated Area 1st	2nd	3rd	4th	Mean (SD)	Testosterone ng/dl in response to GnRH Mean (SD) 1st	2nd	3rd	4th	Mean (SD)
1	G1	PH1 1/1	1.6	0.9	0.8	0.2	1.13 (0.89)	5.0	1.7	1.7	0.3	2.18 (1.73)	1.8	1.3	1.1	1.1	1.33 (0.29)	8.5	6.9	4.7	4.9	6.25 (1.56)	43.8 (6.1)	52.3 (5.2)	44.2 (4.3)	50.2 (3.6)	47.6 (6.0)
2	G1	PH1 1/1	1.6	1.1	1.5	1.3	1.38 (0.19)	6.3	3.6	3.2	3.6	4.18 (1.24)	1.6	1.1	1.5	1.3	1.38 (0.19)	8.7	5.8	4.2	3.1	5.45 (2.11)	15.7 (2.6)	18.7 (3.3)	19.7 (2.4)	20.3 (4.4)	19.0 (3.7)
3	G1	PH1 2/2	4.6	-	-	-	-	16.2	-	-	-	-	2.9	-	-	-	-	14.3	-	-	-	-	27.8 (3.5)	-	-	-	-
4	G1	PH1 2/2	2.5	2.5	2.1	1.9	2.25 (0.26)	11.2	7.3	5.3	4.7	7.13 (2.50)	3.4	2.4	1.6	1.5	2.23 (0.76)	17.9	11.2	5.6	4.8	9.88 (5.25)	60.7 (8.5)	68.0 (6.2)	59.3 (7.4)	56.0 (4.2)	61.0 (8.0)
5	G1	PH1 2/2	2.6	2.3	2.1	2.0	2.25 (0.23)	9.0	7.9	6.1	5.5	7.13 (1.40)	1.1	1.4	1.1	1.0	1.15 (0.15)	5.3	6.7	3.7	4.1	4.95 (1.17)	21.3 (3.0)	32.7 (5.4)	34.7 (6.0)	39.3 (6.0)	32.0)6.6)
6	G1	PH1 3/3	5.8	4.1	3.9	4.5	4.58 (0.74)	17.8	10.9	8.8	10.8	12.1 (3.40)	0.7	0.7	0.5	0.6	0.63 (0.08)	3.7	2.9	1.8	2.1	2.63 (0.74)	23.8 (6.5)	26.7 (2.9)	26.5 (4.3)	26.7 (4.9)	25.9 (5.0)
7	G1	PH1 3/3	9.1	7.6	-	-	-	35.2	26.8	-	-	-	2.1	1.3	-	-	-	9.2	9.0	-	-	-	144 (25)	177 (23)	-	-	-
8	G1	PH1 3/3	5.1	4.9	4.5	4.0	4.63 (0.42)	17.7	12.2	12.7	9.5	13.0 (2.96)	0.6	1.0	0.5	0.5	0.65 (0.21)	2.0	5.0	2.1	2.0	2.78 (1.29)	26.8 (2.3)	31.3 (3.1)	31.7 (1.4)	40.7 (4.0)	32.6 (5.8)
4 II	G1	PH1 3/4	7.4	4.2	3.7	3.8	4.78 (1.53)	35.6	16.8	13.5	11.6	19.4 (9.55)	2.1	1.4	0.8	0.5	1.20 (0.60)	10.5	6.8	2.3	1.4	5.25 (3.66)	98.0 (15.9)	102 (9)	102 (11)	102 (17)	101 (14)
9	G1	PH1 4/3	10.0	11.1	5.9	3.6	7.65 (3.00)	35.0	25.3	13.6	8.7	17.7 (13.2)	0.8	0.6	0.4	0.4	0.55 (0.17)	4.0	2.6	0.4	1.6	2.15 (1.32)	87.5 (8.3)	107 (14)	112 (3)	134 (12)	109 (19)
8 II	G2	PH1 4/4	9.2	4.3	4.4	7.5	6.35 (2.10)	33.8	15.4	14.9	21.8	21.5 (7.60)	0.9	0.7	0.4	0.6	0.65 (0.18)	4.1	2.9	1.3	0.4	2.18 (1.43)	130 (13)	135 (8.5)	128 (6.0)	131 (8.0)	131 (9.6)
10	G2	PH1 4/4	7.9	5.1	3.9	4.1	5.25 (1.60)	33.9	15.1	7.8	13.9	17.7 (9.80)	1.6	1.3	0.6	0.3	0.95 (0.50)	3.9	4.7	1.1	0.3	2.50 (1.84)	299 (71)	340 (25)	343 (18)	322 (19)	326 (44)
6 II	G2	PH1 5/5	7.0	5.4	5.3	4.4	5.53 (0.94)	22.9	14.4	14.8	13.5	16.4 (3.80)	0.3	0.4	0.5	0.5	0.43 (0.08)	1.8	0.7	0.7	2.4	1.40 (0.73)	112 (30)	172 (10)	159 (74)	188 (20)	158 (34)
11	G2	PH1 5/5	11.5	7.5	5.8	6.4	7.80 (2.20)	44.0	22.0	14.4	18.8	24.8 (11.4)	2.4	1.7	2.6	1.0	1.68 (0.50)	9.4	7.4	4.2	3.7	6.18 (2.30)	66.2 (12)	94.8 (9.6)	93.5 (9.5)	107 (6.7)	90 (18)
12	G2	PH2 5/5	14.1	9.5	6.9	7.9	9.60 (2.80)	45.1	22.7	12.4	20.9	25.3 (12.1)	0.7	0.6	0.4	0.2	0.48 (0.19)	2.9	3.3	0.9	0.5	1.90 (1.20)	120 (17)	139 (2.2)	123 (16)	132 (18)	129 (7.5)
9 II	G2	PH2 6/5	13.3	9.6	11.3	6.8	10.30 (2.40)	50.8	36.7	26.5	13.2	31.8 (13.8)	1.3	1.0	1.0	0.5	0.95 (0.29)	6.9	3.9	3.2	0.8	3.70 (2.20)	377 (58)	496 (22)	409 (62)	373 (25)	414 (67)
11 II	G3	PH2	16.2	13.4	11.3	8.4	12.30 (2.90)	48.2	30.1	22.9	20.1	30.3 (10.9)	0.8	1.4	1.1	1.2	1.13 (0.2)	1.9	5.3	4.4	2.4	3.50 (1.40)	282 (18)	284 (15)	316 (24)	360 (36)	313 (41)
13	G3	PH2 8/8	9.0	9.9	8.4	7.8	8.80 (0.80)	35.5	26.2	22.3	24.2	27.1 (5.1)	0.6	0.4	0.7	1.8	0.88 (0.54)	-0.1	1.3	2.2	5.3	2.18 (1.98)	543 (70)	579 (45)	529 (24)	587 (70)	562 (60)
12 II	G3	PH3 8/8	19.1	11.8	9.7	11.0	12.90 (3.70)	72.4	26.7	20.4	30.2	37.4 (20.0)	1.1	1.1	0.1	1.2	0.88 (0.45)	5.5	3.5	-0.9	4.9	4.13 (1.14)	369 (51)	404 (30)	342 (20)	366 (38)	370 (36)
14	G3	PH3 8/8	7.2	4.4	3.7	2.5	4.45 (1.72)	31.3	10.9	12.3	4.9	14.9 (9.9)	1.7	1.2	0.7	0.7	1.08 (0.41)	8.0	5.5	2.8	-0.8	4.58 (2.40)	273 (52)	318 (14)	277 (30)	292 (11)	290 (36)
15	G4	PH3 12/12	12.5	-	-	-	-	42.2	-	-	-	-	1.5	-	-	-	-	7.8	-	-	-	-	327 (75)	-	-	-	-
12 III	G4	PH3 10/10	22.1	11.9	10.5	9.6	13.50 (5.00)	64.4	37.3	23.6	23.2	37.1 (16.7)	2.1	1.5	1.0	0.9	1.38 (0.48)	10.4	6.9	2.4	4.4	6.03 (3.00)	459 (49)	498 (21)	459 (18)	416 (37)	458 (29)
13 II	G4	PH4 12/12	7.5	9.2	6.7	9.4	8.20 (1.14)	32.7	22.7	22.7	29.1	26.8 (4.3)	0.9	0.7	0.2	0.4	0.55 (0.27)	4.7	3.7	0.3	1.6	2.58 (1.73)	509 (79)	531 (48)	485 (54)	507 (43)	508 (60)
14 II	G4	PH4 10/10	19.5	9.8	10.0	5.9	11.30 (5.00)	65.6	22.5	19.6	17.9	31.4 (19.8)	1.9	0.9	1.2	0.8	1.20 (0.43)	9.9	4.1	4.5	2.8	5.33 (2.70)	302 (52)	318 (22)	292 (26)	334 (27)	312 (37)
16	G4	PH4 12/12	11.6	10.8	6.7	8.0	9.28 (2.00)	45.4	26.4	19.5	24.8	29.0 (9.8)	1.5	0.8	0.7	0.2	0.80 (0.46)	6.1	3.5	2.3	-0.4	3.08 (2.10)	465 (38)	478 (16)	482 (32)	646 (116)	519 (99)
13 III	G5	PH4 18/15	9.1	8.3	5.8	7.8	15.50 (14.2)	28.7	25.3	14.0	15.6	20.9 (6.2)	0.7	0.5	0.3	0.6	0.53 (0.15)	2.2	2.3	0.7	1.6	1.70 (0.64)	562 (29)	632 (38)	651 (28)	754 (33)	650 (76)
12 IV	G5	PH4 18/15	20.9	12.1	14.1	9.2	14.10 (4.30)	84.9	27.4	35.9	17.6	41.5 (26.0)	1.5	0.8	0.8	1.5	1.15 (0.35)	7.3	2.6	2.9	4.8	4.40 (1.90)	576 (72)	625 (44)	584 (15)	681 (89)	617 (55)
14 III	G5	PH5 12/12	11.8	7.2	5.8	3.8	7.15 (2.90)	40.9	17.8	17.7	12.8	22.3 (10.9)	1.2	1.1	0.7	0.5	0.88 (0.29)	9.7	4.0	2.8	2.3	4.70 (2.95)	347 (18)	363 (32)	352 (21)	381 (29)	361 (29)
16 II	G5	PH4 15/15	20.5	13.0	14.2	12.5	15.10 (3.20)	75.9	34.8	35.8	34.4	45.2 (18.0)	1.0	0.7	0.4	0.4	0.63 (0.25)	5.1	2.4	1.1	0.5	2.30 (1.80)	474 (31)	548 (57)	593 (72)	553 (77)	542 (75)
16 III	G5	PH5 20/20	17.8	8.4	9.9	11.2	11.80 (3.60)	60.7	27.2	19.6	21.8	32.3 (17.0)	0.8	0.5	0.2	0.4	0.50 (0.22)	3.9	2.6	0.2	1.9	2.15 (1.30)	485 (31)	511 (33)	478 (20)	538 (44)	503 (40)

Table 4.1

Mean (SD) Hormone Concentration	Time Period	PUBERTY STAGE 1A N=5	1B N=5	2 N=6	3 N=4	4 N=5	5 N=5	Linearity	Slope or Stage Effect
LH u/l	E	0.87 (0.23)	1.39 (0.65)	2.11 (0.96)	2.40 (0.90)	3.52 (0.74)	3.31 (0.68)	yes	p<0.001
	N	1.13 (0.54)	3.01 (1.24)	4.92 (1.13)	5.18 (1.54)	5.76 (1.32)	5.52 (0.94)	no	p<0.001
	M	0.90 (0.27)	2.08 (0.78)	3.08 (0.88)	4.62 (0.74)	3.97 (0.54)	3.95 (0.75)	no	p<0.001
FSH u/l	E	1.50 (0.55)	1.71 (0.51)	1.91 (0.50)	2.15 (0.23)	2.65 (0.43)	2.67 (0.77)	yes	p<0.001
	N	1.54 (0.56)	1.78 (0.45)	2.16 (0.63)	2.36 (0.42)	2.74 (0.44)	2.62 (0.80)	yes	p<0.001
	M	1.58 (0.54)	2.04 (0.68)	2.36 (0.67)	2.90 (0.68)	3.02 (0.53)	2.91 (0.88)	yes	p<0.001
Testosterone ng/dl ml	E	35.5 (13)	37.4 (20)	68.6 (46)	160 (109)	225 (39)	272 (37)	yes	p<0.001
	N	34.8 (11)	64.9 (31)	197 (102)	335 (126)	435 (76)	478 (118)	yes	p<0.001
	M	38.5 (17)	82.2 (49)	206 (131)	390 (99)	452 (73)	510 (105)	yes	p<0.001
Oestradiol pg/ml	E	22.3 (8)	22.6 (4)	22.3 (5)	28.4 (7)	24.2 (6)	25.2 (3)	yes	NS
	N	20.7 (5)	22.7 (3)	32.0 (11)	28.8 (5)	26.5 (4)	27.5 (4)	yes	NS
	M	21.9 (6)	23.4 (5)	33.1 (8)	31.5 (6)	24.6 (4)	29.3 (4)	no	p<0.001
Prolactin mu/l	E	325 (324)	442 (230)	248 (101)	180 (64)	262 (98)	262 (51)	yes	NS
	N	412 (55)	580 (237)	500 (149)	332 (88)	631 (291)	800 (375)	yes	NS
	M	234 (59)	358 (217)	274 (106)	330 (102)	430 (144)	472 (178)	yes	p<0.05

Table 4.2a

The mean (SD) LH, FSH, testosterone, oestradiol and prolactin in the evening (E) 20.00-23.00 h, night (N) 23.00-07.00 h and morning (M) 07.00-09.00 h in 30 studies from 16 pubertal subjects classified into six clinical stages of puberty (1A-5). The relationships of the mean hormone levels with pubertal stages are expressed in terms of linearity and the slope of the linear relationship or a general stage effect if the relationship is non-linear.

	Time Period	Mean (SD) Pulse Frequency/10 hours Amplitude u/l	PUBERTY STAGE 1A	1B	2	3	4	5	Linearity	Slope or Stage Effect
LH	Evening	Frequency	0	0	0.6 (1.2)	2.5 (1.4)	4.7 (1.6)	4.7 (1.6)	Yes	p<0.001
		Amplitude	0	0	0.90	1.07 (0.12)	2.27 (0.93)	2.87 (1.47)	Yes	p<0.01
	Night & Morning	Frequency	0.8 (0.8)	3.0 (1.1)	4.7 (0.5)	4.3 (0.8)	4.0 (0.6)	4.2 (0.8)	No	p<0.001
		Amplitude	0.88 (0.27)	1.82 (0.66)	2.43 (0.41)	3.11 (0.77)	3.91 (0.96)	4.31 (1.24)	Yes	p<0.001

Table 4.2b LH pulse frequency and amplitude in the evening (20.00-23.00 h) and night + morning (23.00-09.00 h) in 30 studies from 16 pubertal subjects classified into 6 clinical stages of puberty (1A-5). The relationships of the mean hormone levels with pubertal stages are expressed in terms of linearity and the slope of the linear relationship or a general stage effect if the relationship is non-linear.

Mean ± (SD) GnRH response			PUBERTY STAGE 1A	1B	2	3	4	5	Linearity	Slope or Stage Effect
LH	Δ Max u/l	First pulse	2.58 (1.10)	7.48 (1.87)	10.50 (2.66)	12.88 (4.93)	14.64 (5.37)	16.02 (4.75)	yes	p<0.001
		Mean of 4 pulses	1.75 (0.51)	5.41 (1.30)	7.47 (1.94)	9.60 (3.37)	10.60 (2.00)	12.70 (3.10)	yes	p<0.001
		Mean of 2nd to 4th pulses	1.56 (0.15)	4.95 (1.03)	6.44 (0.32)	8.53 (1.02)	9.05 (0.98)	9.55 (0.47)	yes	p<0.001
	Integrated Area Area units	First pulse	9.54 (4.00)	28.26 (8.58)	38.40 (9.20)	46.85 (16.00)	50.06 (12.90)	58.22 (20.99)	yes	p<0.001
		Mean of 4 pulses	5.16 (2.10)	15.50 (3.60)	22.90 (5.16)	27.40 (8.10)	31.10 (3.80)	32.40 (9.80)	yes	p<0.001
		Mean of 2nd to 4th pulses	4.25 (0.66)	13.60 (3.56)	17.73 (2.47)	20.94 (1.80)	24.11 (2.41)	23.86 (2.55)	yes	p<0.001
FSH	Δ Max u/l	First pulse	2.16 (0.85)	1.26 (0.69)	1.20 (0.68)	1.05 (0.42)	1.58 (0.41)	1.04 (0.29)	no	p<0.05
		Mean of 4 pulses	1.52 (0.40)	0.76 (0.30)	0.86 (0.42)	0.99 (0.11)	0.98 (0.33)	0.74 (0.25)	no	p<0.05
		Mean of 2nd to 4th pulses	1.37 (0.13)	0.68 (0.22)	0.74 (0.18)	0.97 (0.24)	0.78 (0.16)	0.63 (0.10)	no	p<0.001
	Integrated Area Area units	First pulse	10.94 (4.50)	5.88 (3.33)	4.80 (2.56)	3.85 (3.10)	7.78 (2.18)	5.64 (2.62)	no	p<0.01
		Mean of 4 pulses	6.63 (1.93)	3.20 (1.20)	2.98 (1.60)	3.60 (0.91)	4.26 (1.46)	3.05 (1.24)	no	p<0.01
		Mean of 2nd to 4th pulses	5.48 (1.54)	2.90 (1.67)	2.36 (1.06)	3.13 (0.63)	3.04 (1.07)	2.18 (0.51)	no	p<0.01

Table 4.2c

The LH and FSH response (maximal incremental and integrated response area) to four 10 µg GnRH bolus stimulation in 30 studies from 16 pubertal subjects classified into 6 clinical stages of puberty (1A-5). The relationships of the mean hormone levels with pubertal stages are expressed in terms of linearity and the slope of the linear relationship or a general stage effect if the relationship is non-linear.

of four profiles from one subject over a period of 27 months (Fig 4.2a-c).

The earliest detectable change in this series of studies was the nocturnal rise in plasma LH concentrations associated with sleep (Figs 4.1 & 4.2 and Appendix II). This was observed before there was any clinical evidence of pubertal onset in terms of testicular enlargement to 4 mls or more and pubic hair development. At this time, FSH concentrations were invariably higher than that of LH. Early puberty was characterized by small amplitude and high frequency nocturnal LH pulsatile secretion with considerable shifts of the baselines values during the night compared with morning or evening. In late puberty, higher amplitude but lower frequency LH pulses were observed in the night as well as during the evening. However, the difference in baseline concentrations of LH between the sleep and awake periods was relatively small. Although a distinct pulsatile pattern of FSH secretion was not consistently present at any stage, most patients showed a progressive elevation of plasma FSH concentrations during the night with peak values being achieved in the morning.

Both LH and FSH secretions were stimulated by the repeated exogenous GnRH injections in all subjects. The LH response increased with pubertal maturation but no obvious potentiation or exhaustion of the pituitary responsiveness was elicited by the two-hourly frequency of GnRH stimulation. Subjects in stage 1A tended to have higher FSH responses than those in later stages of development. Testosterone concentrations increased

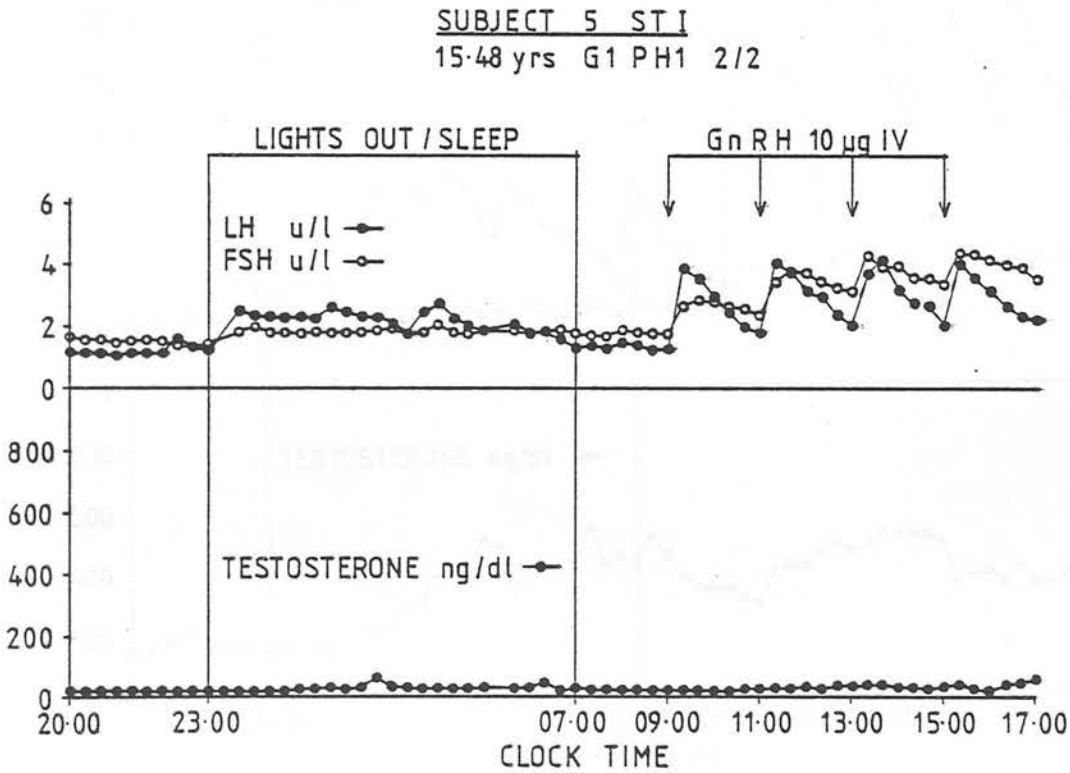


Fig 4.1a

Profiles of plasma LH, FSH and testosterone in subject 5 ST I representative of late prepuberty. The hormone concentrations were determined at 20-minute intervals for 21 hours. The pituitary and testicular responsiveness was assessed by four GnRH (10 µg) intravenous bolus injections at two-hourly intervals.

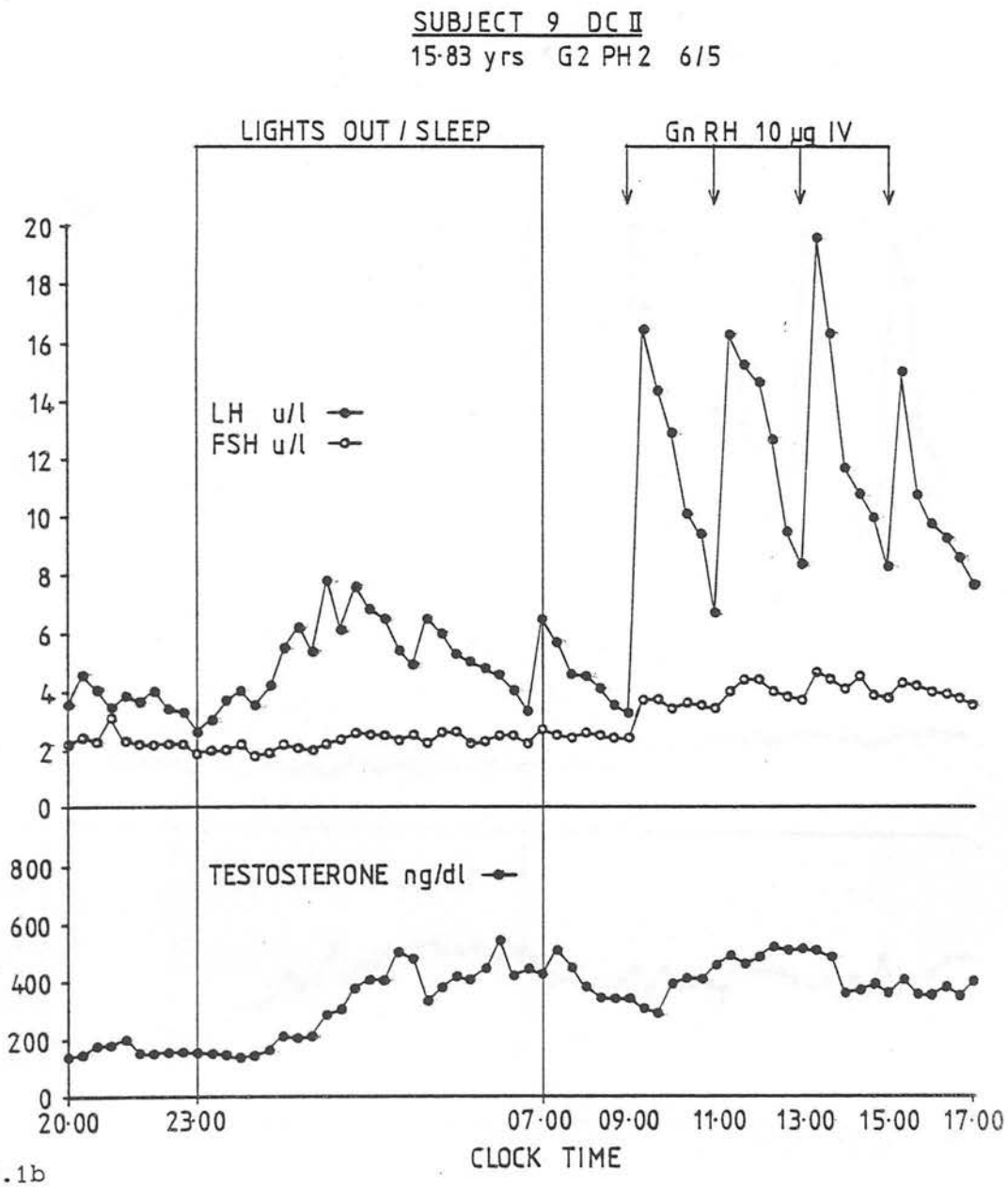


Fig 4.1b

Profiles of plasma LH, FSH and testosterone in subject 9 DC II representative of mid-puberty. The hormone concentrations were determined at 20-minute intervals for 21 hours. The pituitary and testicular responsiveness was assessed by four GnRH (10 µg) intravenous bolus injections at two-hourly intervals.

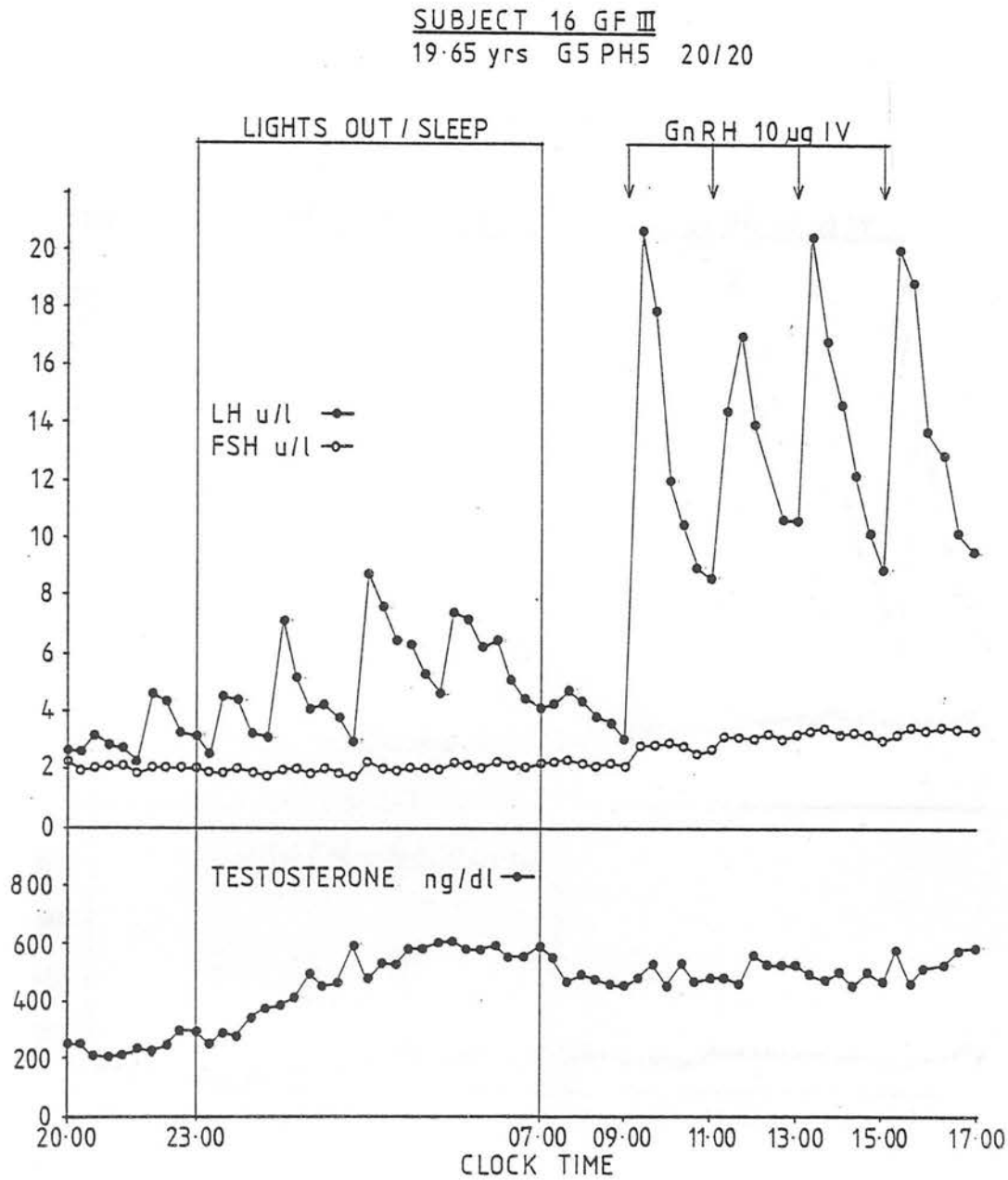


Fig 4.1c

Profiles of plasma LH, FSH and testosterone in subject 16 GF III representative of late puberty. The hormone concentrations were determined at 20-minute intervals for 21 hours. The pituitary and testicular responsiveness was assessed by four GnRH (10 µg) bolus injections at two-hourly intervals.

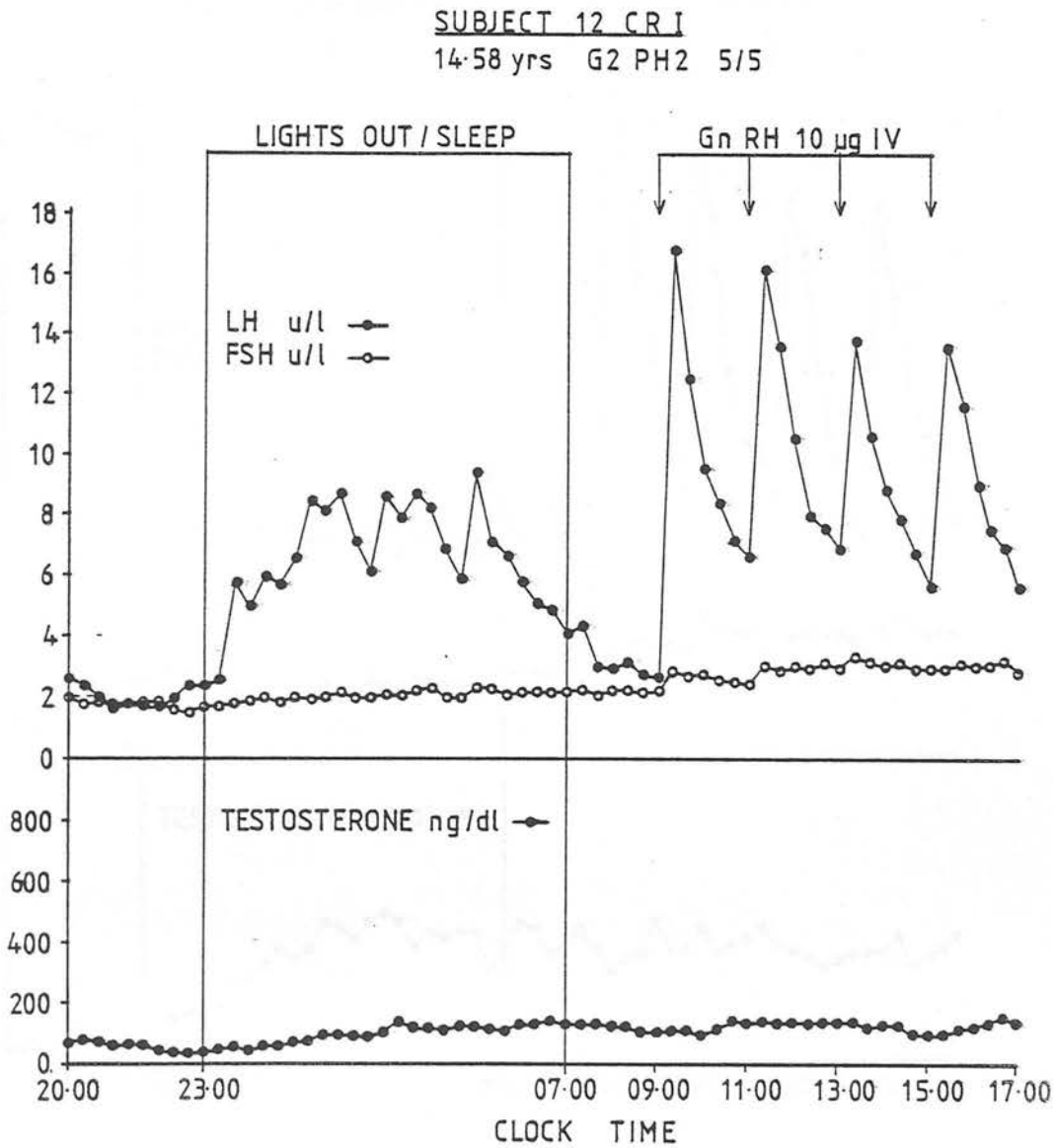


Fig 4.2a

Profiles of plasma LH, FSH and testosterone in one subject (subject 12 CR) studied in early puberty over a period of 12 months. The hormone concentrations were determined at 20-minute intervals for 21 hours. The pituitary and testicular responsiveness was assessed by four GnRH (10 µg) intravenous bolus injections at two-hourly intervals.

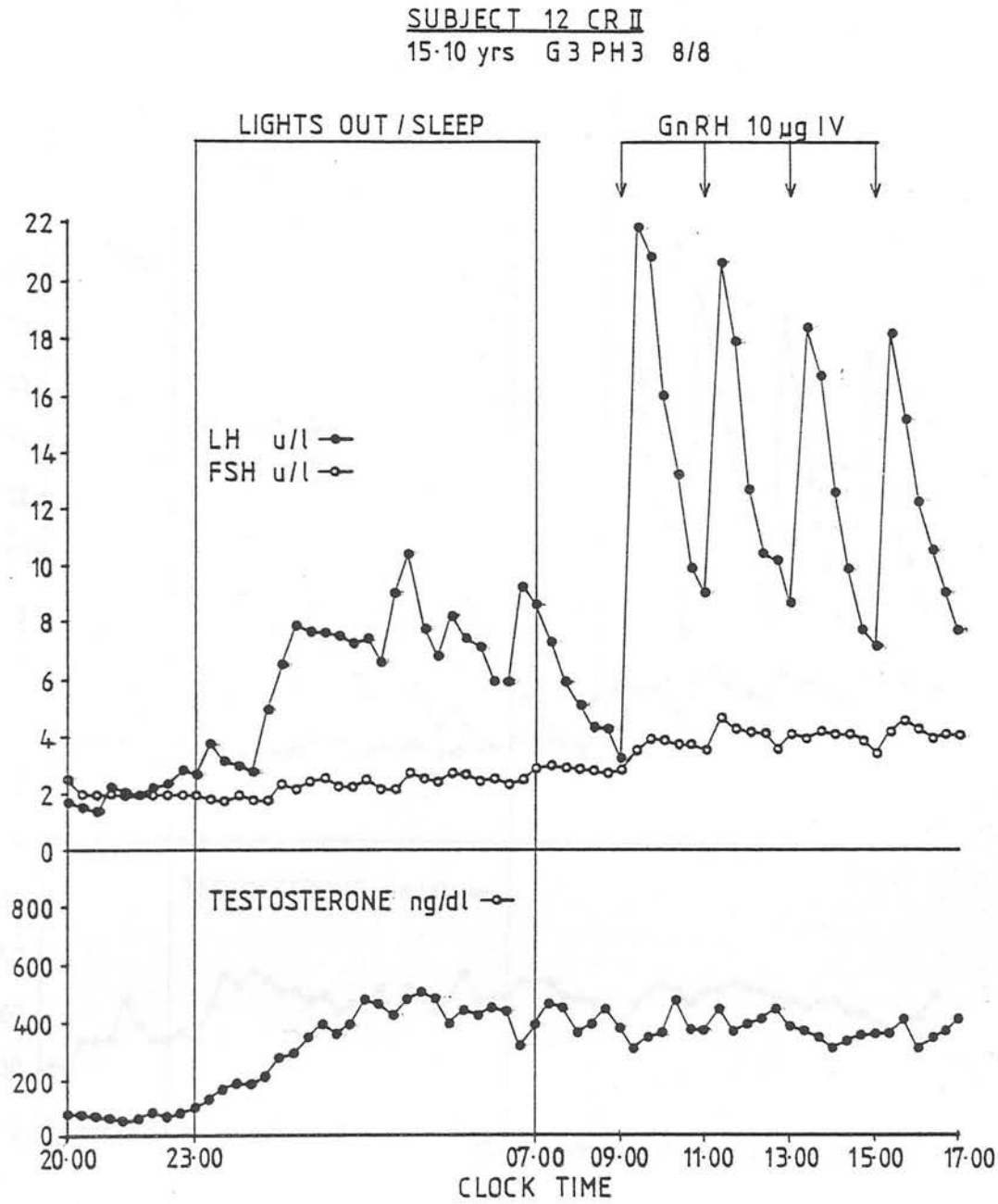


Fig 4.2b

Profiles of plasma LH, FSH and testosterone in one subject (subject 12 CR) studied in mid puberty over a period of 12 months. The hormone concentrations were determined at 20-minute intervals for 21 hours. The pituitary and testicular responsiveness was assessed by four GnRH (10 µg) intravenous bolus injections at two-hourly intervals.

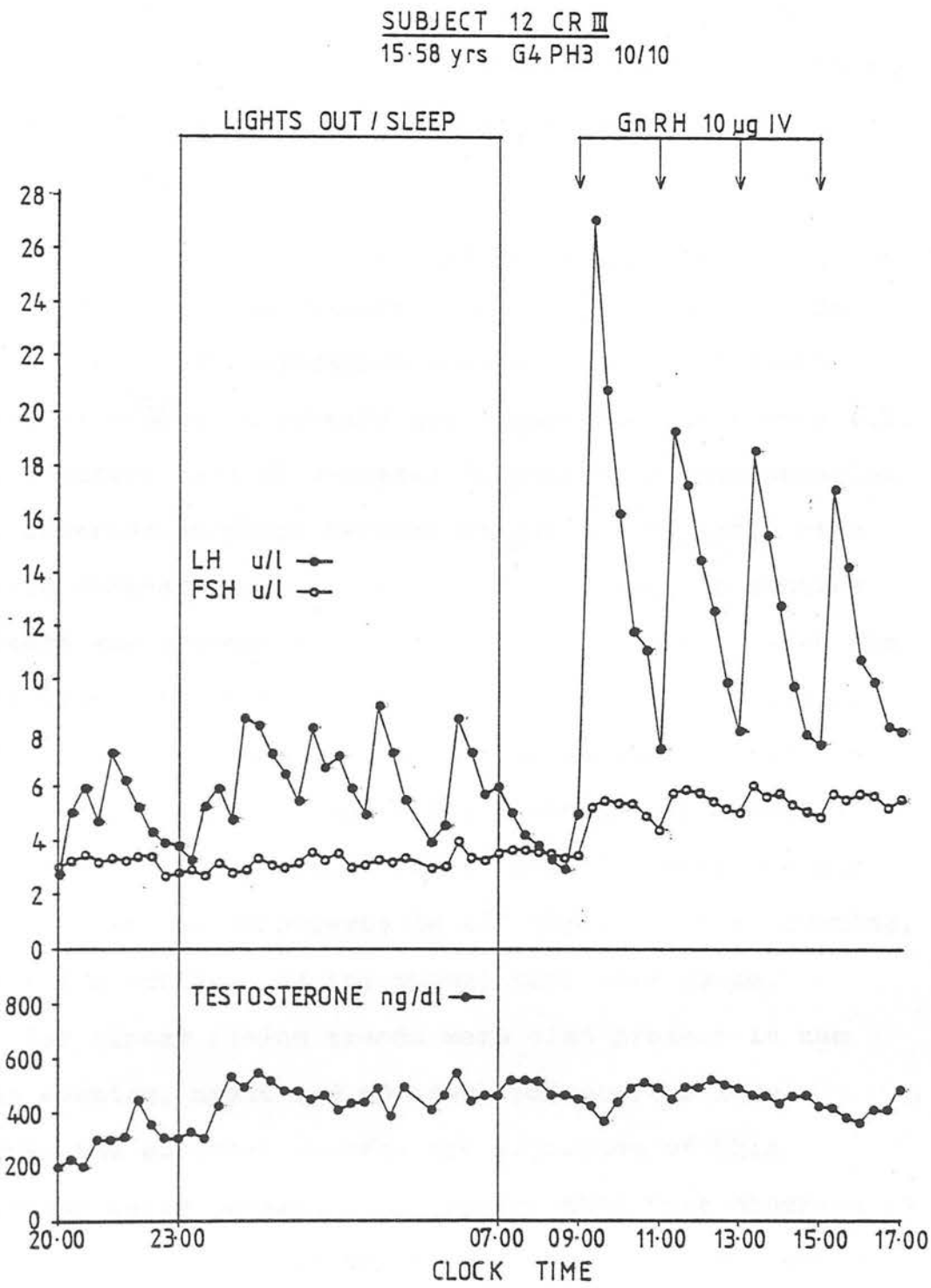


Fig 4.2 c

Profiles of plasma LH, FSH and testosterone in one subject (subject 12 CR) studied in late puberty over a period of 12 months. The hormone concentrations were determined at 20-minute intervals for 21 hours. The pituitary and testicular responsiveness was assessed by four GnRH (10 µg) intravenous bolus injections at two-hourly intervals.

during the night from early puberty onwards but adult levels were not reached until late puberty. No distinct pulsatile pattern of testosterone secretion was seen in most subjects.

4.2.2 Mean diurnal plasma hormone concentrations

Changes in mean plasma concentrations of LH, FSH, testosterone and oestradiol across the six different clinical stages of puberty are illustrated in Figure 4.3. The greatest rate of increase in plasma LH concentration was observed at night between stages 1A, 1B and 2 with little further change in subsequent stages. A similar pattern was present in plasma LH in the morning with the peak levels being achieved at stage 3. A more gradual and linear rise in plasma LH concentration was seen in the evening. Mean plasma FSH concentrations showed a significant linear rising trend ($p<0.001$) over the six clinical stages of puberty in all three periods (evening, night and morning) of the twenty-four hour cycle. Similar linear rising trends were also present in the mean evening, night and morning testosterone levels across the pubertal stages, the magnitude of this increase being several fold greater than that observed in gonadotrophins. Adult concentrations of plasma testosterone were reached by stage 3 or 4 in some patients in the morning or night but somewhat later in the evening. Oestradiol levels remained low (20-40 pg/ml) throughout puberty with no discernible trends in any part of the day or night.

The diurnal changes in mean plasma concentrations of

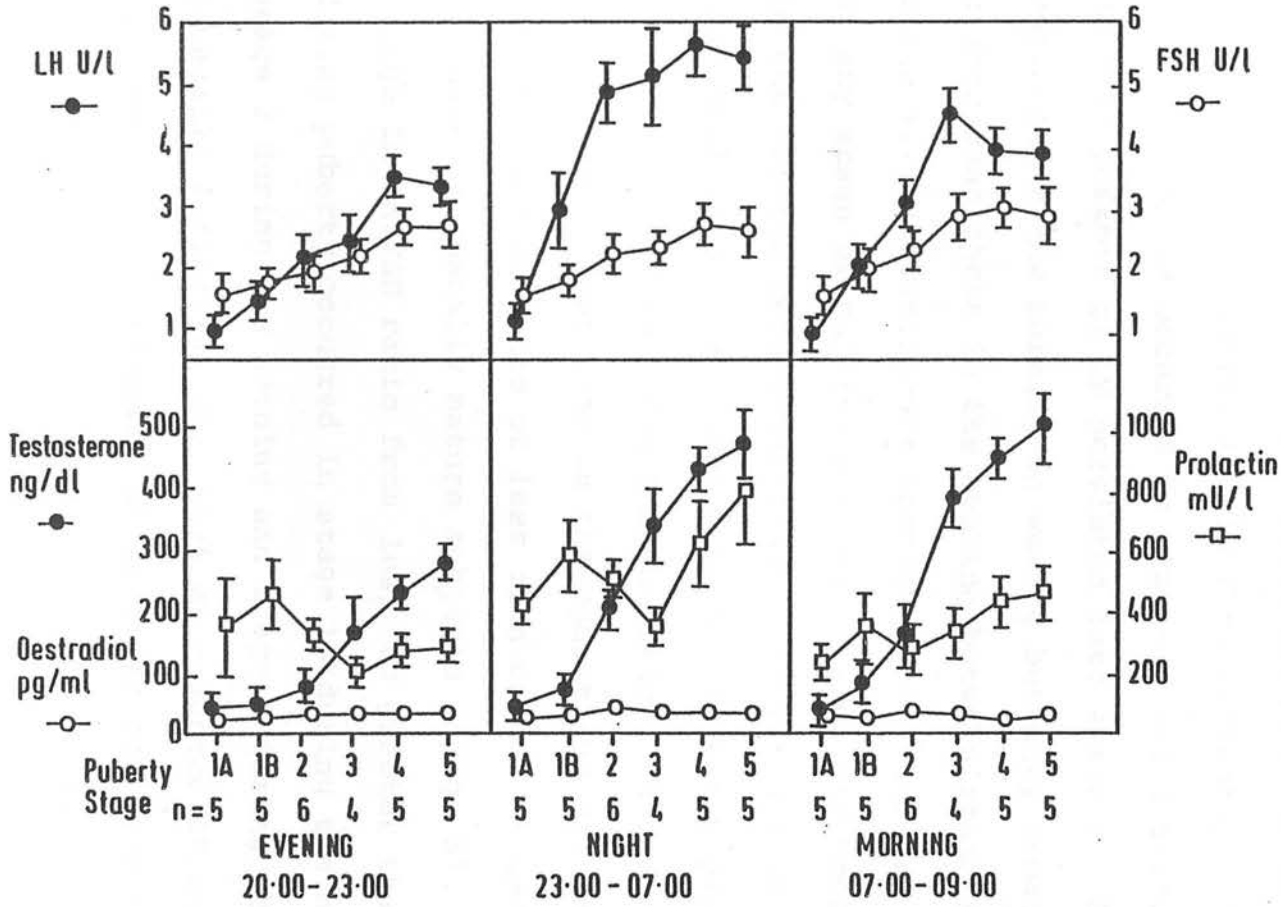


Fig 4.3

Mean (SEM) plasma LH, FSH, prolactin, testosterone and oestradiol in the six stages of puberty (see Chapter 3) at three different parts of the 24-hour cycle: Evening (E) 20.00-23.00 h, night(N) 23.00-07.00 h and morning (M) 07.00-09.00 h.

LH, FSH, testosterone and oestradiol in each of the six clinical stages of puberty are shown in Figure 4.4. Significant nocturnal elevation of LH was first encountered in stage 1B. This constituted the earliest detectable hormonal change which heralds the onset of puberty. The greatest absolute or percentage increase in nocturnal LH occurred in stages 2 and 3 although the diurnal pattern of LH persisted into stage 5. LH levels declined in the morning on waking but they remained higher than those in the evening between stage 1B to stage 5. No significant nocturnal FSH rise was observed at any stage of puberty but mean plasma FSH concentrations in the morning were significantly elevated from the nocturnal levels in stages 2 to 5. In early prepubertal subjects (stage 1A), FSH concentrations in peripheral plasma were higher than LH throughout the day and night with the LH:FSH ratio of less than one. The reverse was the case in sexually mature subjects (stage 5). The change in LH:FSH ratio from less to greater than one during puberty occurred in stage 1B during the night, stage 2 during the morning and stage 3 during the evening, generally reflecting the onset of enhanced LH secretion with pubertal development at specific portions of the twenty-four hour cycle. The diurnal pattern of testosterone during puberty showed nocturnal increases becoming detectable from stage 1B onwards and persisting into stage 5. Unlike LH whose concentrations fell on waking in the morning, the elevated nocturnal levels of testosterone were maintained or might even increase

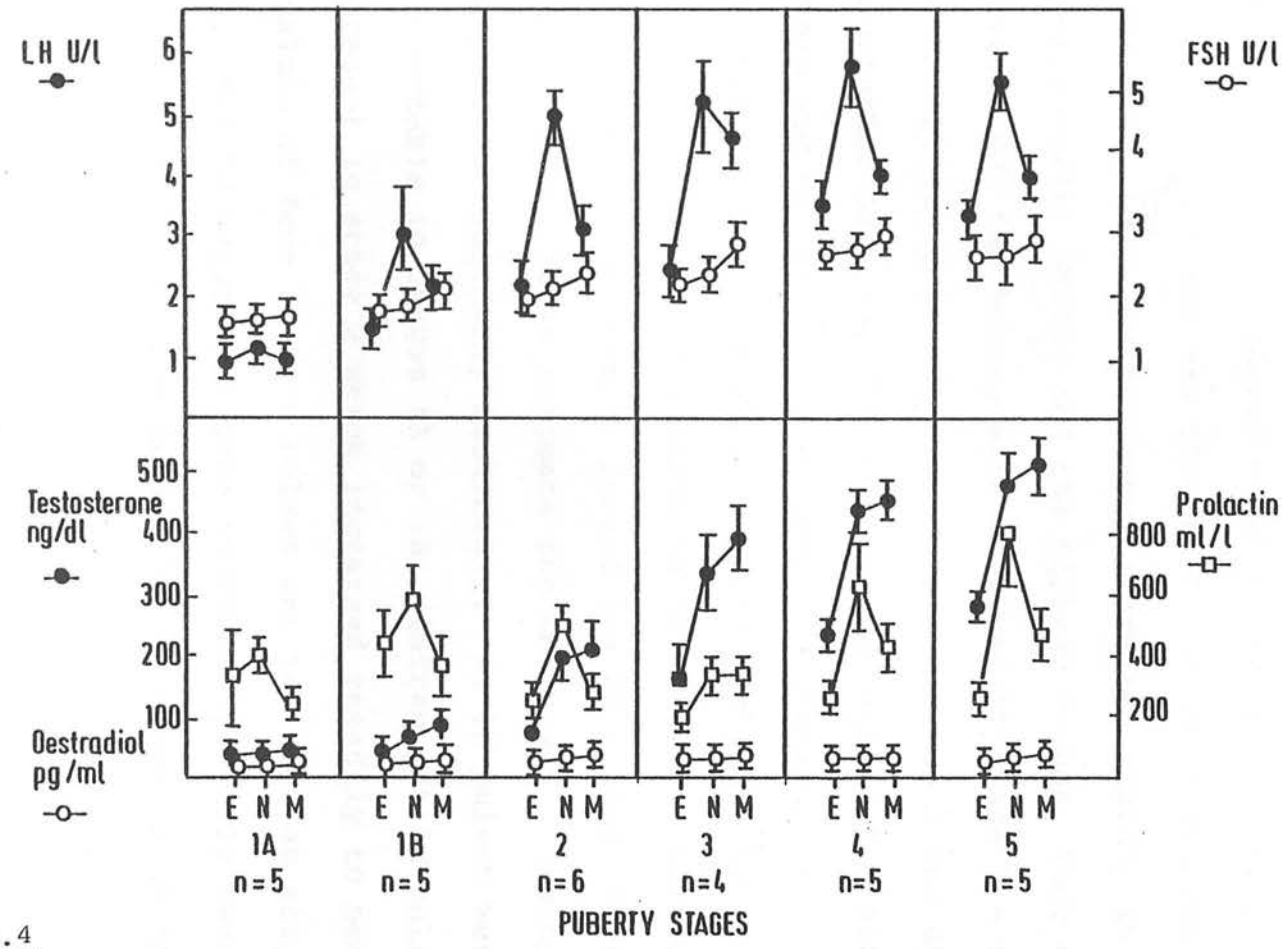


Fig 4.4

The diurnal pattern of mean (SEM) plasma LH, FSH, prolactin, testosterone and oestradiol in the six stages of puberty (see Chapter 3). Evening (E) 20.00-23.00 h, night (N) 23.00-07.00 h, and morning (M) 07.00-09.00 h.

further. Oestradiol displayed a consistent lack of diurnal variation during puberty.

4.2.3 Pulsatile LH secretion

The frequency and amplitude of LH secretion during puberty are shown in Figure 4.5. The morning and night periods were considered together as the duration of the former on its own was too short for pulse analysis. Nocturnal LH pulse frequency increased rapidly in the peripubertal period and the highest values, four to five pulses per ten hours, were achieved at stage 2 - the clinical onset of puberty. Between stage 2 and stage 5, nocturnal LH pulse frequency became attenuated although this did not reach the 5% level of statistical significance. Nocturnal LH pulse amplitude increased in a linear fashion from stage 1A to stage 5. Although the duration of the evening period was only three hours, it proved possible to estimate the LH pulse frequency and amplitude reasonably accurately. No LH pulses were detectable in stages 1A or 1B. Infrequent LH pulses were present in stage 2 which increased steadily to peak values of four to five pulses per ten hour at stage 4 and 5. The LH pulse amplitude increased linearly between stages 2 and 5. Thus pulsatile LH secretion in the evening, and possibly for the rest of the waking hours, followed a pattern similar to the nocturnal period from which it lagged behind by some two clinical stages of puberty.

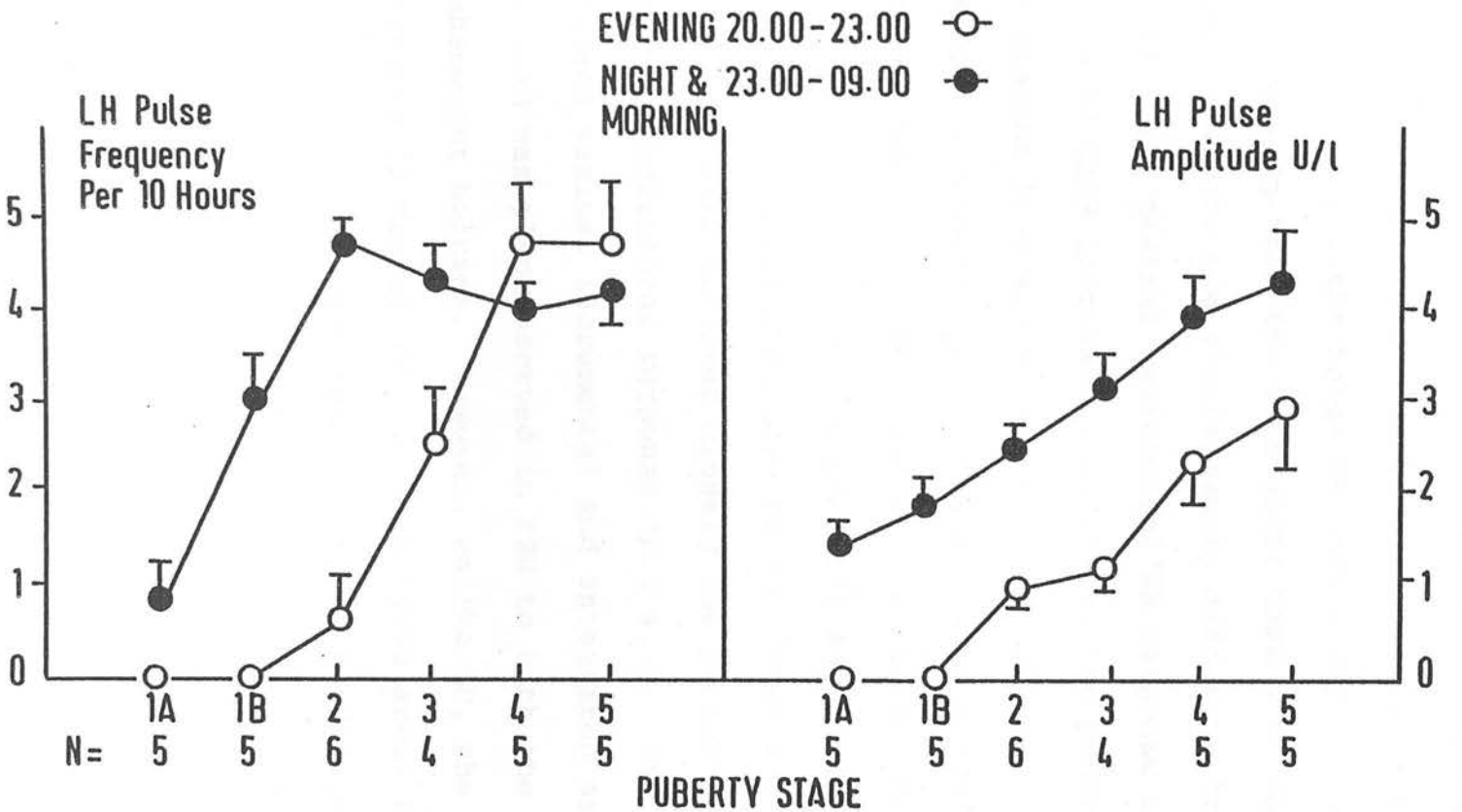


Fig 4.5

Mean (SEM) LH pulse frequency per 10 hours and LH pulse amplitude during pubertal stages 1A-5 in the evening (20.00-23.00 h) and night combined with morning (23.00-09.00 n).

4.2.4 Pituitary responsiveness to GnRH

A significant pituitary LH and FSH response could be elicited by the present submaximal but repeated GnRH stimulation regime in all subjects including the most sexually immature individuals (Fig 4.1, Appendix II). For the maximal incremental or integrated area of LH response, the first bolus of GnRH always produced a greater rise than the subsequent three, to which the responses were not significantly different from each other. The maximal incremental LH response to the first bolus of GnRH increased steadily during pubertal maturation from stage 1A onwards (Fig 4.6). The mean maximal incremental LH response to the second, third and fourth GnRH bolus demonstrated a parallel increase to the first response across the pubertal stages (Fig 4.6). The integrated LH response area to the first and subsequent pulses of GnRH followed closely the pattern of the maximal incremental response (Fig 4.7). The similarity between maximal incremental and integrated area response to GnRH was also observed in FSH to both the first and subsequent boluses. However, unlike LH, the maximal FSH response (Δ Max as well as integrated area) to exogenous GnRH stimulation was seen in stage 1A, following which the magnitude of response was diminished and remained unchanged in the subsequent stages of puberty (Fig 4.7).

The dynamics of LH secretion elicited by exogenous GnRH stimulation in the six stages of puberty is summarized in Table 4.3. The apparent half-lives of circulating LH were calculated individually from the

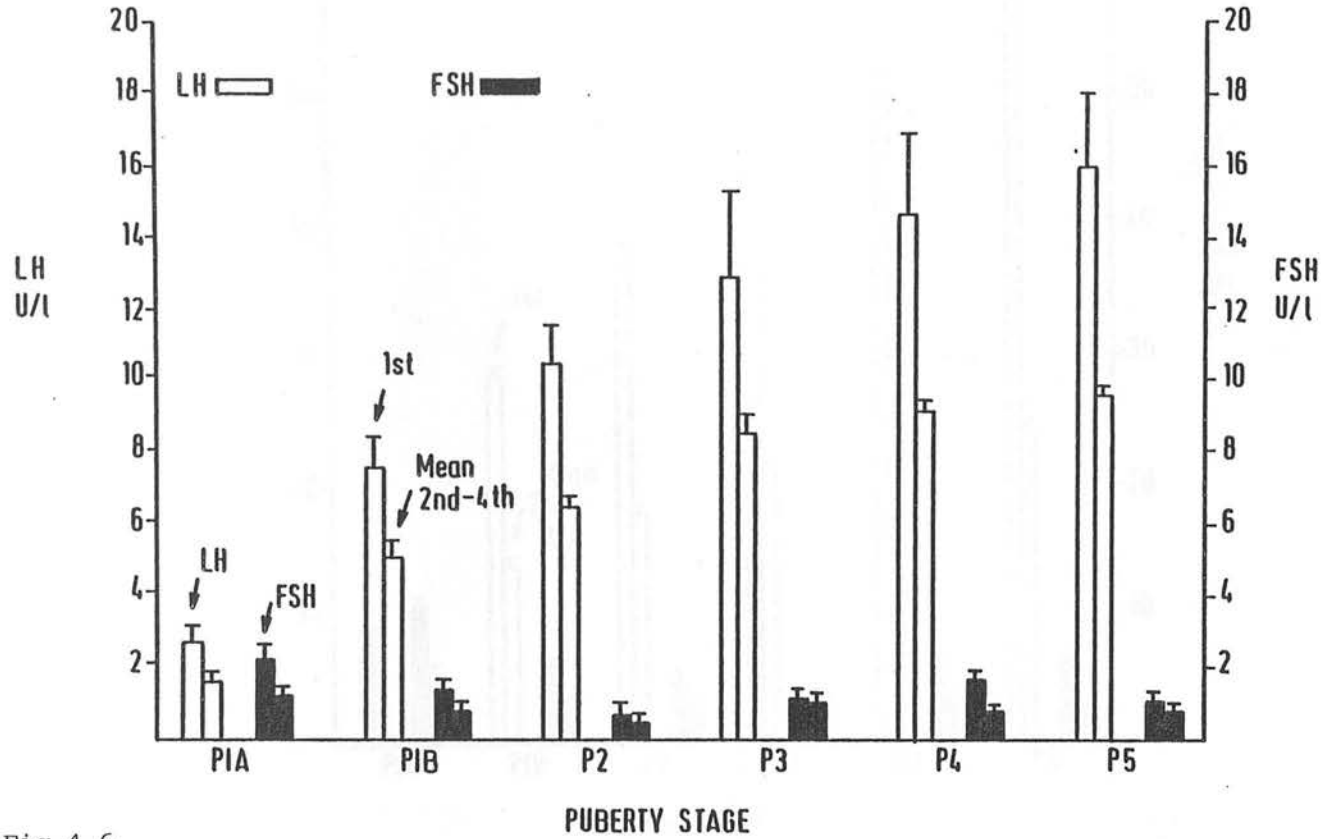


Fig 4.6

Mean (SEM) maximal incremental LH and FSH response to the first and the average response to the second, third and fourth GnRH (10 µg) I.V. bolus stimulation in puberty stages 1A-5.

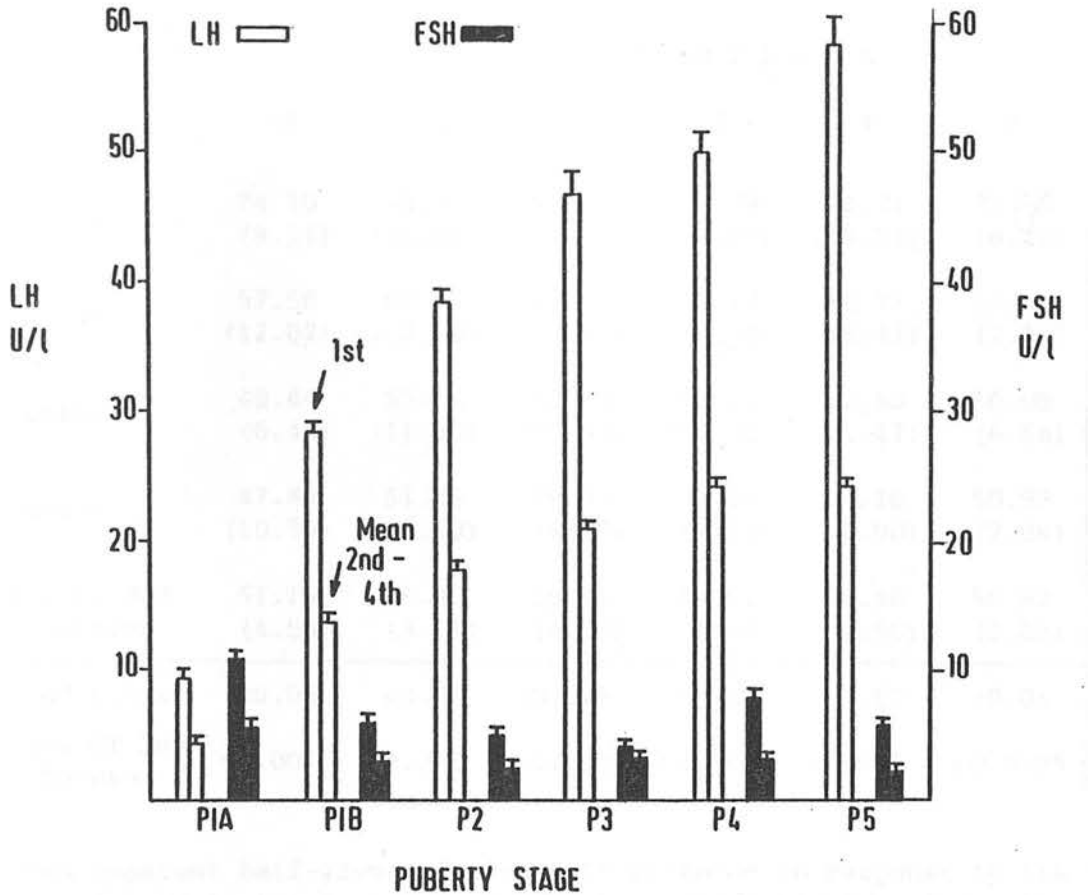


Fig 4.7

Mean (SEM) integrated LH and FSH response area to the first and the average response to the second, third and fourth GnRH (10 µg) I.V. bolus stimulation in puberty stages 1A-5.

		PUBERTY STAGES						
		1A	1B	2	3	4	5	statistical difference
Apparent half-life of LH secreted in response to GnRH 10 µg bolus	1st bolus	74.10 (9.31)	74.87 (11.81)	73.60 (7.36)	75.29 (9.97)	71.71 (10.01)	71.18 (6.12)	N.S.
	2nd bolus	57.58 (12.02)	59.82 (13.18)	61.18 (9.95)	48.17 (3.30)	51.71 (6.47)	54.80 (7.18)	N.S.
	3rd bolus	48.44 (6.44)	55.16 (11.20)	49.35 (10.44)	50.55 (10.41)	52.53 (11.17)	50.05 (6.84)	N.S.
	4th bolus	47.46 (10.35)	51.22 (5.68)	56.30 (8.98)	49.91 (1.24)	54.16 (2.90)	50.92 (2.88)	N.S.
	Mean of 2nd, 3rd and 4th boluses	51.16 (4.56)	55.40 (3.51)	55.61 (4.85)	49.91 (1.24)	54.16 (2.90)	50.92 (2.88)	N.S.
Statistical difference p	1st vs 2nd bolus	<0.05	<0.01	<0.025	<0.01	<0.05	<0.05	
	1st vs mean of 2nd to 4th boluses	<0.005	<0.005	<0.0005	<0.005	<0.005	<0.0005	

Table 4.3

The mean (SD) calculated apparent half-lives of plasma LH secreted in response to the four successive 10 µg bolus of GnRH stimulation in the 6 stages of puberty. Statistical analysis is described in Section 2.7. Not significant (N.S.) - $p>0.05$.

integrated LH response area to each bolus of GnRH divided by the maximal LH increment and multiplied by twenty minutes. The mean apparent half-lives of circulating LH secreted in response to the pulsatile exogenous GnRH stimulation remained remarkably constant during sexual maturation irrespective of the increasing magnitude of pituitary response. In each of the six stages of puberty, mean apparent half-life of LH in the first induced response was significantly greater than the subsequent three, whose LH apparent half-lives were very similar. Thus the relationship between the first and subsequent three responses to pulsatile GnRH stimulation has remained constant throughout puberty.

4.2.5 Endogenous GnRH pulsatile secretion

Since the qualitative aspects of pituitary LH secretion in response to exogenous GnRH have been shown to remain relatively constant during pubertal development, it may be assumed that the pituitary responded to endogenous GnRH in a similar manner. With knowledge of both the nocturnal spontaneous LH pulse amplitude induced by endogenous GnRH pulses as well as the LH pulse amplitude (Δ Max) produced by exogenous GnRH pulses in the same subject, it was possible to estimate the magnitude of endogenous GnRH pulses using a liner log-dose relationship (Kelch et al, 1975; Wollensen et al, 1976). Injection of a 10 µg bolus of GnRH into the antecubital vein would produce a concentration of GnRH in the hypophysial circulation which was dependent on the blood/plasma volume of distribution - 8.7% of body weight

		PUBERTY STAGE					
		1A	1B	2	3	4	5
Magnitude of endogenous pulsatile GnRH secretion pg/ml	Mean	32.5	16.6	16.3	20.8	19.0	33.8
	SD	22.9	10.4	15.5	13.6	9.4	43.1
	Range	2.4-57.8	4.2-30.9	1.6-49.2	6.7-38.5	7.5-34.6	4.5-118.5
	N	3	5	6	4	5	5

Table 4.4

The estimated GnRH concentration in the pituitary portal circulation from endogenous hypothalamic pulsatile secretion in the six stages of puberty.

(Kelch et al, 1975) provided there was no immediate metabolism or preferential uptake by the hypothalamus. The concentration of exogenous GnRH in the hypophysial circulation (a) could then be used to calculate the endogenous nocturnal GnRH concentration (b) by relating to the mean Δ Max LH response to exogenous GnRH (y) and the mean nocturnal LH pulse amplitude (x). Thus the estimated endogenous GnRH concentration, Log b = Log a x x/y. The estimated peak concentrations of endogenous nocturnal GnRH pulses secreted into the hypophysial portal circulation in the six stages of puberty are shown in Table 4.4. There was no significant change in the magnitude of hypothalamic GnRH pulsatile secretion during pubertal development. The estimated range of GnRH concentration in the hypophysial portal circulation varied from 1.6-119 pg/ml.

4.3 Discussion

The present results confirmed the pubertal increase in plasma concentrations of gonadotrophins and testosterone described in previous studies based on day-time samples (Burr et al, 1970; August et al, 1972; Winter & Faiman, 1972; Faiman & Winter, 1974; Lee et al, 1974; Baker et al, 1976). The presence of nocturnal sleep-related gonadotrophin and testosterone secretion in puberty was also confirmed in boys with delayed puberty (Boyar et al, 1972b & 1973). Amongst these earlier studies, however, conflicting data on the timing and magnitude of the various hormonal changes were present. Thus, Faiman & Winter (1974) found

that plasma FSH rise preceded that of LH while Lee et al (1974) demonstrated the reverse. The precise determination of temporal relationships in LH and FSH secretion in puberty is important in advancing the understanding of the mechanism of puberty and the initiation of spermatogenesis with respect to the augmented Leydig cell steroidogenesis. Furthermore, how the nocturnal sleep-related gonadotrophin and testosterone secretions fit into the day-time pattern of hormonal changes with advancing sexual maturation has not been systematically studied.

Our results demonstrated that the pattern of LH and FSH secretion, particularly in early puberty, varied considerably depending on the clock-time of blood sampling (Figs 4.3 & 4.4). A recent study (Beck & Wuttke, 1980) in normal children aged 1-15 years has also yielded very similar results. The earliest and also the greatest rate of LH increase occurred at night while the morning samples showed FSH levels at their maximum although the rate of FSH rise through puberty at different parts of the day were not different. The diurnal variation in LH and to a lesser extent FSH may account for some of the discrepancies in previous studies based on single blood samples taken at different or unspecified times of the day. The difficulty in interpretation of pubertal hormone data without taking into account the important nocturnal period is self-evident.

Since the weight of evidence at present favours the existence of a single hypothalamic GnRH which stimulates

the pituitary release of both LH and FSH (Schally et al, 1976), the dissociated pattern of LH and FSH secretion during puberty, especially highlighted in the nocturnal period, must result from differences in dynamics of pituitary secretion of the two gonadotrophins and the longer half-life of FSH in the circulation (Yen et al, 1970). Nocturnal LH secretion was characterized by the acute rise and fall of plasma LH concentration compatible with its shorter circulating half-life (Yen et al, 1968; Santen & Bardin, 1973) and the episodic stimulation of its release by pulsatile endogenous GnRH secretion of the hypothalamus. The FSH secretory pattern showed a much slower rise and fall in plasma concentrations with small but more sustained peak levels which may not return to baseline before the start of the next secretory episode. Both patterns of endogenous gonadotrophin secretion were also well demonstrated by the contrasting response patterns of LH and FSH to repetitive exogenous pulsatile GnRH stimulation at different stages of maturation (Figs 4.1 & 4.2 & Appendix II). The stepwise upward shift in baseline FSH concentrations produced by exogenous GnRH pulses resulted in peak values being attained at the end of the stimulation period. This same pattern of pituitary FSH secretion can probably account for the observed increase in plasma FSH from night to morning in response to nocturnal endogenous GnRH pulsatile stimulation. These results and their interpretations are consistent with similar observations on the pituitary response to repetitive GnRH stimulation in humans (Reitano et al,

1975; Krabbe & Shakkebaek, 1977), rams (Licoln, 1979) and rats (Johnson & Mellampiani, 1975).

This study also confirmed that in early prepubertal and hypogonadotrophic subjects, stable but low circulating levels of LH and FSH were characteristic. In addition, FSH concentrations were consistently higher than those of LH in sexually immature patients, being the reverse of the adult pattern. The first detectable change indicating the onset of pubertal development was the presence of nocturnal pulsatile GnRH/gonadotrophin secretion coinciding with the reversal of the LH:FSH ratio to greater than unity at night in stage 1B (Figs 4.3 & 4.4). This reversal of the LH:FSH ratio occurred one or two stages later in the morning and evening, again temporally correlated with the later appearance of pulsatile GnRH secretion at these times. It is reasonable therefore to suggest that the reversal of plasma concentrations of LH and FSH during puberty may be the result of an increasing frequency and/or amplitude of pulsatile GnRH stimulation of the pituitary gonadotropes producing larger, acute but brief LH secretory episodes in contrast to the smaller, more sluggish but longer-sustained FSH secretion. Recent studies in the ovariectomized monkeys bearing hypothalamic lesions showed that lowering the frequency of pulsatile GnRH stimulation from one pulse per hour to one pulse per three hours produced a disproportionately large FSH response compared to LH and a reversal of the LH:FSH ratio (Wildt et al, 1981). It appears that at lower

pulse frequencies, the effect of the 3-fold longer FSH half-life is exaggerated although this may not be the only explanation. Whatever the mechanism, that study emphasized the importance of frequency modulation of pituitary gonadotrophin secretion on the absolute and relative concentrations of circulating LH and FSH. Apart from the obvious parallel in the pattern of gonadotrophins in puberty as demonstrated in this study, similar changes in LH:FSH ratio have also been observed in other physiological or pathological situations such as recovery from post-lactational or postpartum amenorrhoea (Rolland et al, 1975; Plant et al, 1980) and in the polycystic ovarian syndrome (Yen, 1980).

In women with gonadal dysgenesis or after the menopause and in adult castrates of both sexes, plasma FSH concentrations are usually in excess of LH despite the presence of normal or even enhanced pulsatile GnRH stimulation (Yen et al, 1972a & b; Root et al, 1972; Conte et al, 1975). This implies that a negative feedback signal from the gonad that preferentially suppresses pituitary FSH secretion must normally exist. This is compatible with the finding that maximal FSH responsiveness to exogenous GnRH is present in early prepubertal subjects (stage 1A) which rapidly became attenuated in stage 1B with the first sign of testicular enlargement. The exaggerated FSH response to GnRH during the earliest part of sexual maturation has also been observed recently in hypogonadotrophic patients treated by low-dose pulsatile GnRH administration (Jacobson et al,

1979; Valk et al, 1980) or by a superactive GnRH analogue (Crowley et al, 1980a). In some of these cases, especially females, a decline in FSH response to GnRH was observed after the initiation of treatment. It is obvious that there is little increase in testicular steroid secretion at this early stage to account for the enhanced negative feedback. On the other hand, the establishment of sertoli cell function, seminiferous tubular growth and germ cell differentiation are believed to take place in early puberty (Steinberger et al, 1978; Richardson & Short, 1979). It seems likely that the negative feedback of pituitary FSH secretion established in early puberty is effected by seminiferous tubular factors. The seminiferous tubules at this stage of development are unlikely to contain any germ cells more mature than early spermatids (Sniffen, 1950; Charny et al, 1952; Albert et al, 1953) so that the source of the tubular negative feedback signal for FSH must reside in either the Sertoli cells or the immature germ cells. This is consistent with the findings of A. Steinberger (1979) which suggested that Sertoli cells in culture can elaborate a factor which inhibited pituitary cell FSH release. The relative importance of gonadal negative feedback versus the frequency modulation of GnRH stimulation in producing the change in pattern of LH and FSH secretion in puberty remains to be clarified. However, it is interesting to note that despite the early attenuation of pituitary FSH responsiveness to GnRH, mean FSH levels in subsequent pubertal stages continued to

rise until late puberty (Fig 4.9). This could possibly reflect the relatively minor role in the negative feedback control of FSH contributed by the seminiferous tubules compared to the later and more significant effects of testicular steroids (Morris & Jackson, 1978).

The change in frequency and amplitude of pulsatile LH secretion is one of the crucial events in pubertal development. This study has clearly demonstrated that the appearance of sleep-related episodic LH secretion is the earliest detectable change in hypothalamic-pituitary function. From the low and stable baseline concentrations of LH in stage 1A, maximal LH pulse frequency of 4.7 ± 0.5 pulses per ten hours or a pulse interval of 128 ± 12 minutes was attained as early as stage 2, indicating the early functional maturity of the hypothalamus at what is clinically the onset of puberty. Thereafter, nocturnal LH pulse frequency showed a tendency to decline although this did not reach the 5% level of statistical significance (see later). Nocturnal LH pulse amplitude however demonstrated a progressive five-fold increase from stage 1A to 5. These changes were associated with an increase in mean nocturnal LH from stage 1A to stage 2, whereupon no subsequent rise occurred - a pattern closely paralleled to that of nocturnal LH pulse frequency rather than amplitude (Fig 4.9). This is not unexpected considering the short circulating half-life of LH. Thus changes in the mean concentration of this hormone are rarely seen without concurrent alterations in the frequency of episodic LH secretion (Knobil, 1981). Increase in LH pulse

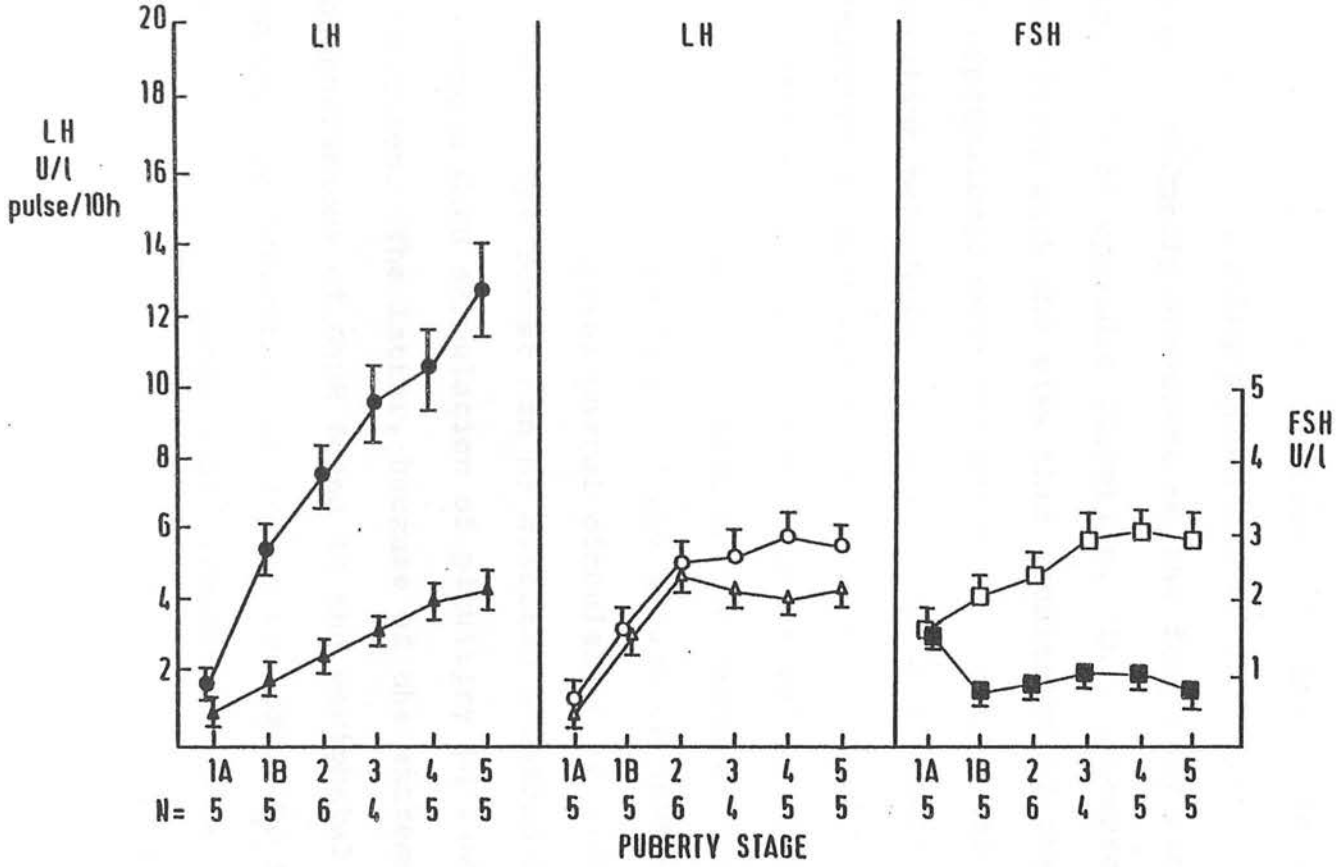


Fig 4.9

The dynamics of gonadotrophin secretion during puberty. The maximal LH incremental response to 10 μg of GnRH I.V. —●—, and the nocturnal LH pulse amplitude —▲— increased by 7 and 5-fold respectively from stage 1A to stage 5. Mean nocturnal LH concentration —○— , and nocturnal LH pulse frequency —△— increased only from stage 1A to stage 2 with no further rise thereafter. Mean FSH incremental response to 4 consecutive 10 μg doses of GnRH I.V. at 2 hourly intervals —■— was maximal in stage 1A but the mean morning plasma FSH —□— increased steadily from stage 1A to stage 3.

amplitude may not be so critical provided the secretory pulse interval is not appreciably shorter than the duration of a secretory episode. This point is well demonstrated by the relatively stable mean LH concentration in puberty stages 4 and 5 despite the continuing increase in LH pulse amplitude. It can be inferred that the mean circulating concentration of LH during puberty is predominantly dependent on the frequency rather than amplitude of episodic secretion. This interpretation is consistent with the view that modulation of the frequency of hypothalamic GnRH secretion is probably the most important mechanism regulating the development of pituitary gonadotrope function during puberty.

The progressive increase in LH pulse amplitude in puberty is mainly dependent on two factors: the pituitary responsiveness to GnRH and the amount of GnRH released into the hypophysial portal circulation in each secretory episode. The former can be directly assessed by exogenous GnRH stimulation of pituitary LH (and FSH) production. The latter, because of the extremely low concentrations of GnRH found in the peripheral circulation, can only be estimated indirectly by comparing the exogenously and endogenously-induced LH pulse amplitudes. The present results failed to show any progressive increase in the magnitude of hypothalamic pulsatile GnRH secretion during puberty with mean values in the six clinical pubertal stages varying from 15-35 pg/ml. The range of estimated GnRH concentrations (1.6-119 pg/ml) is in the same order of magnitude as that obtained by direct

measurement of GnRH in hypophysial portal blood in adult Rhesus monkeys (Carmel et al, 1976; Neil et al, 1977) and rats (Sarkar et al, 1976; Eskay et al, 1977) which yielded values varying from undetectable to 1000 pg/ml. Kelch's group, using a similar approach to ours, estimated the pituitary portal GnRH concentration in human pubertal and adult males to be between under 30-300 pg/ml (Kelch et al, 1975; Huseman & Kelch, 1978; Corley et al, 1981). In the adult Soay ram, the estimated magnitude of hypothalamic GnRH secretion increased and subsequently declined during the transition from the regressed to the active stages of the seasonal reproductive cycle with pituitary portal GnRH concentrations between 60-217 pg/ml. These estimates of GnRH secretion are based on the assumptions that injected GnRH is uniformly distributed in the circulation, that it was not immediately metabolized or preferentially taken up by the hypothalamus and that the concentration of GnRH in the systemic venous circulation is equivalent to that in blood entering the hypophysial portal vessels. It is further assumed that exogenous and endogenous GnRH evoked qualitatively similar resonses in the pituitary throughout the twenty-four hours. Despite these possible sources of inaccuracy, gross underestimation of endogenous GnRH production is unlikely since the twenty-minute sampling frequency in the present study tends to underestimate the peak LH response to exogenous GnRH stimulation. Support for the absence of a pubertal rise in GnRH pulse amplitude is also provided by the

concurrent seven-fold increase in pituitary responsiveness to GnRH. The latter can more than account for the five-fold rise in nocturnal LH pulse amplitude during puberty without the need to invoke any changes in magnitude of GnRH secretion (Fig 4.9). It appears that in man the pubertal rise in LH pulse amplitude is the direct result of the maturation of pituitary gonadotropes with the concomitant enhanced responsiveness to GnRH stimulation. These changes of the pituitary may be induced by the priming effect of increased frequency of hypothalamic GnRH stimulation. Support for this is found in patients with Kallmann's syndrome (congenital deficiency of hypothalamic GnRH) and other disorders of HPT axis where prolonged treatment with exogenous GnRH can restore normal responsiveness in the pituitary (Yoshimoto et al, 1975; Hashimoto et al, 1975). It can be said that the increase in both frequency and amplitude of pulsatile LH secretion in puberty are directly or indirectly related to the increased frequency of hypothalamic GnRH stimulation.

The availability of synthetic GnRH and miniaturized programmable infusion pumps has facilitated the treatment of hypogonadotrophic hypogonadism by the pulsatile administration of small doses of GnRH (Jacobson et al, 1979; Crowley & McArthur, 1980a; Valk et al, 1980). Although the early results are favourable, the dose of GnRH administered varied from 25 ng/Kg body weight to 40 μg per pulse. Since unphysiological doses of GnRH may produce negative effects on both pituitary and testicular

functions (Linde et al, 1981; Comite et al, 1981), it is important to optimize the dose regime of pulsatile GnRH treatment of hypogonadotrophic patients. Based on the results of this study, the dose of exogenous GnRH administered which produces physiological concentrations of GnRH and pituitary responses similar to spontaneous GnRH pulses in puberty is 92 ng/Kg body weight or 3.8 µg per bolus. Thus the administration of around 4 µg of GnRH intravenously at one to two hourly intervals should theoretically yield optimal pituitary-testicular responses with minimal inhibitory effects on prolonged treatment.

Closer scrutiny of the individual hormonal profiles disclosed a further aspect of nocturnal LH secretion which was not apparent from the meaned data (Figs 4.1, 4.2 & Appendix II). In the early pubertal profiles, the nocturnal rise in LH took the form of frequent but small amplitude secretory episodes producing a marked and sustained upward shift of the LH baseline concentration. However, due to the short interval between LH secretory episodes and the comparatively long sampling interval of twenty minutes, the exact frequency of nocturnal LH secretory episodes tended to be underestimated in this study. The mean nocturnal LH concentrations in early to mid puberty, despite the much smaller amplitude of individual LH pulses, were comparable to later stages. This implies that the nocturnal LH pulse frequency in early to mid puberty must be significantly higher than can be gauged from the present methods of sampling or analysis. From puberty stage 4 onwards, the nocturnal LH

secretory pattern showed a distinct but gradual change to less frequent LH pulses with greater amplitude each of which returned towards baseline before the onset of the next secretory episode. This is reminiscent of the response pattern to the two-hourly exogenous GnRH stimulation and suggests that the frequency of endogenous GnRH pulses during sleep in early puberty must be considerably in excess of one every two hours. The pattern of LH secretion in the evening was similar to the nocturnal period except that the high frequency and low amplitude pulsatile secretion of early puberty was absent or undetectable. Since the evening LH pulse amplitudes were comparatively small even at stage 5, it is possible that any low amplitude fluctuations in plasma LH concentration in early puberty would have been below the sensitivity of the assay.

It has already been mentioned that low-dose pulsatile GnRH administration by programmed infusion pumps could induce the "maturation" of the pituitary gonadotrophin secretory pattern in patients with hypogonadotrophic hypogonadism. It may therefore be inferred that pulsatile GnRH stimulation at two hourly intervals in the doses used may be able to emulate the functional maturation of the pituitary and testes in spontaneous puberty. However, the duration of these studies are relatively short and final conclusions must await longer-term results. Experimental studies in monkeys and lambs (Knobil et al, 1980b; Steiner & Bremner, 1981; Foster et al, 1978; Ryan & Foster, 1980) have unequivocally

confirmed that GnRH stimulation at the optimal pulse frequency for these species of one hourly was successful in inducing the full development of the immature gonads. Foster et al (1978) also showed that GnRH/LH pulse frequency became attenuated at the peripubertal period in the ram-lamb. This observation suggested that the pubertal changes in functional dynamics of the hypothalamic-pituitary testicular axis may involve additional factors other than a simple feedforward cascade mechanism initiated by an increase in pulsatile GnRH secretion. This is compatible with the pattern of LH/GnRH secretion observed in human puberty in this study. Thus the barely detectable levels of LH in prepuberty was succeeded by the high frequency and low amplitude fluctuations in early puberty which then gave way to the lower frequency and high amplitude LH secretory pattern in late puberty and adulthood. It may be speculated that the high frequency GnRH secretion at the onset of puberty is a unique ontogenic phenomenon in humans transiently entrained to CNS activities such as sleep. Once pubertal development is under way and the pituitary gonadotropes have developed adequate synthetic capacities in mid-puberty, the endogenous hypothalamic rhythm (two-hourly pulses) manifests itself predominantly while the high frequency sleep-related rhythm is either lost or submerged. In this formulation, sleep entrainment of GnRH secretion can be regarded as a neuroendocrine mechanism which man has phylogenetically evolved to trigger the onset of puberty at an appropriate stage of physical and

CNS maturity. The only other situation where sleep-related gonadotrophin secretion is encountered is in the active phase of anorexia nervosa (Boyar et al, 1974b) where the loss of body mass is said to mimic the reversal of changes during pubertal development and thus allows the re-emergence of sleep-related gonadotrophin secretion. Sleep-associated gonadotrophin secretion, to the best of the author's knowledge, has not been described in any other species apart from humans. A recent report described the presence of diurnal variation in the pulsatile LH secretion of intact male adult rhesus monkeys (Plant et al, 1981) but the relationship to the sleep-wake cycle was not ascertained.

There is evidence for a different though not mutually exclusive explanation for the observed changes in the pattern of pulsatile GnRH/LH secretion during pubertal development. In the adult male, LH pulse frequency averaged about one per two hours (Nankin & Troen, 1971; Santen & Bardin, 1973; Huseman & Kelch, 1978) which is more or less similar to that found in late pubertal subjects in this study. In adult premenopausal females, frequent (one pulse per ninety minutes) small amplitude LH pulses typify the follicular phase while in the mid and late luteal phase, low frequency (one pulse per two to six hours) and high amplitude pulses are characteristic (Yen et al, 1974). This change in pulsatile pattern of LH secretion, not unlike that seen during puberty, is attributed to the feedback effects of oestradiol and progesterone on the hypothalamic-

pituitary unit during the latter half of the menstrual cycle (Yen et al, 1975). It is possible that in the presence of relatively stable levels of testosterone, the LH secretory pattern in the adult male is intermediate between the follicular and luteal phase in the female. In postmenopausal or agonadal females and those with gonadal dysgenesis, LH pulse frequency is not different from that in the follicular phase of menstruating women - the high mean gonadotrophin concentrations resulting from increased pulse amplitude exclusively (Yen et al, 1972 a & b; Root et al, 1972). This implies that physiological concentrations of oestradiol in the female have minimal effects on LH pulse frequency - a finding supported by the feedback studies of Wallach et al (1973) and Rosenfield et al (1973). By analogy, the high frequency GnRH secretion in early male puberty may be equivalent to the follicular phase in premenopausal females and as the testes mature, testosterone feedback gradually reduces the GnRH pulse frequency. That the negative feedback of testosterone has a preferential or selective effect on LH pulse frequency while leaving pulse amplitude unchanged or even enhanced was clearly demonstrated by Santen (1977) and Winters et al (1979). Furthermore, the pituitary response to GnRH stimulation during testosterone infusion remained unchanged (Santen, 1977). These findings together with those in this study strongly support the crucial role of testosterone in modifying the frequency of hypothalamic GnRH pulsatile secretion in the latter half of pubertal development. The major weakness of this

hypothesis is that it does not explain the sleep-entrained nature of gonadotrophin secretion in early and mid-puberty nor does it elucidate the mechanism of the delayed onset of GnRH secretion during the day.

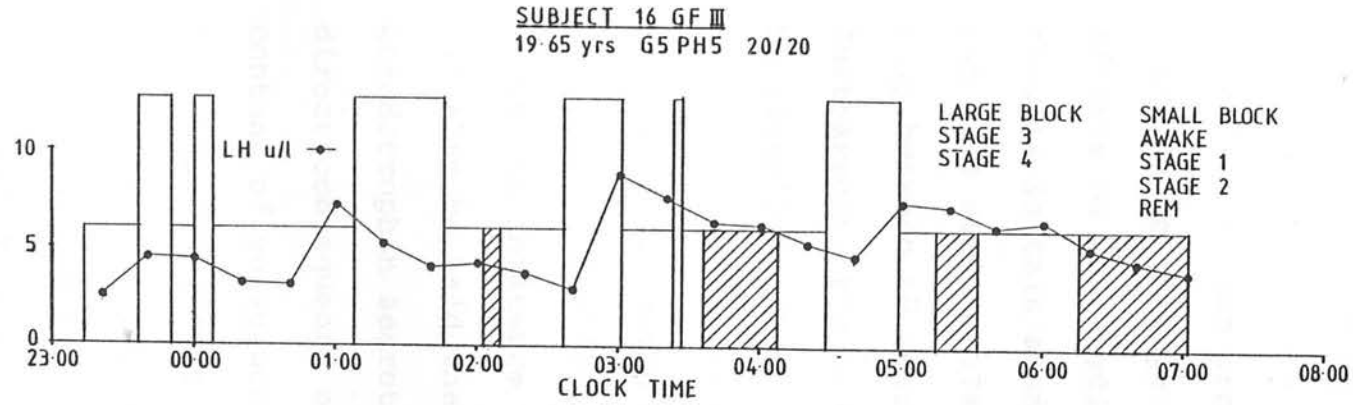
From the foregoing discussion, it is evident that neither sleep-entrained GnRH secretion nor the gonadal feedback mechanisms individually can fully account for the evolution of the pattern of pulsatile gonadotrophin secretion during puberty. It is conceivable that both mechanisms combine to produce the changes observed in this study. The onset of puberty may be triggered by the high frequency GnRH discharges which, by some unknown mechanisms, require the specific neurohumoral environment provided by the state of sleep in the maturing brain. Further development of the CNS after the onset of puberty presumably allows the facilitatory neurohumoral ambience to be maintained throughout the twenty-four hours and GnRH secretion can be stimulated throughout the sleep-wake cycle. Presently, testicular maturation has also advanced to the extent that the increasing circulating concentrations of testosterone or other gonadal secretory products were capable, by negative feedback, of further modifying the pattern and amount of GnRH and gonadotrophin sythesis and release. Thus the GnRH pulse frequency is slowed while the magnitude of LH secretory episodes continue to increase until a new equilibrium is achieved in late puberty which is maintained in adulthood. The role of testosterone in puberty, apart from the initiation of spermatogenesis and induction of secondary

sexual characteristics, may be to modify the rate of GnRH secretion so that the pituitary and gonads do not become desensitized by the increasing levels of GnRH (Clayton & Catt, 1981). Curiously, the relatively simple study of examining the pulse frequency and amplitude of LH secretion in pre and post-pubertal male castrates has not yet been performed.

A close relationship between specific sleep stages and gonadotrophin secretion was suggested by the correlation between the number of LH secretory episodes and the number of sleep cycles - four to six per night on average in adults. The LH secretory pulse interval of 90 minutes also matches the 80-90 minute periodicity of the REM (rapid eye movement) and non-REM sleep cycle (Weitzman et al, 1975). These authors further suggested that LH secretion was initiated during non-REM sleep and terminated during or close to REM sleep in pubertal boys and girls (Boyar et al, 1972b). There is evidence that non-REM sleep is initiated and maintained by serotoninergic mechanisms and REM sleep is initiated by serotoninergic but maintained by noradrenergic and cholinergic mechanisms (Jouvet, 1969). The same neurotransmitters are also involved in the control of hypothalamic GnRH secretion (McCann & Ojeda. 1976). It has been speculated that neurotransmitter functions may provide the chemical basis linking sleep stages and gonadotrophin secretion (Boyar, 1978). However, the effect of serotonin on GnRH secretion and LH secretion is inhibitory (Gallo & Moberg, 1977) while noradrenaline is

generally regarded as a stimulator of pulsatile GnRH secretion (Bhattacharya et al, 1972; Drouva & Gallo, 1976). It is therefore not surprising that a direct relationship between LH secretion and specific sleep stages in pubertal subjects has not been confirmed (Parker et al, 1975). In the present study, the onset of LH secretory episodes was randomly distributed between REM, stage I & II and III & IV sleep (Fig 4.8). Similar findings in adult men have also been reported (Yen et al, 1974; Alford et al, 1973) although the study of Rubin et al (1972) showed a 14% higher concentration of plasma LH during REM than non-REM sleep. Collectively, these results suggest that the transient temporal association between the rest phase of the sleep-wake cycle (rather than specific sleep stages) and GnRH/LH secretion may be due to certain common neural mechanisms which are maturity-determined and may involve changes in neuro-transmitter functions. The further elucidation of the neurohumoral basis of sleep-related pubertal mechanisms will have to depend on neuropharmacological studies designed to investigate the relationships between sleep-wake cycle, neuroendocrine secretions and biological rhythm control.

The secretion of growth hormone is closely linked to slow-wave (stage III & IV) sleep (Takashashi et al, 1968; Honda et al, 1969; Parker et al, 1969; Sassin et al, 1969). Growth hormone has widespread effects on protein, carbo-hydrate and fat metabolism and is considered to be one of the principle anabolic hormones (Korner, 1965). This has



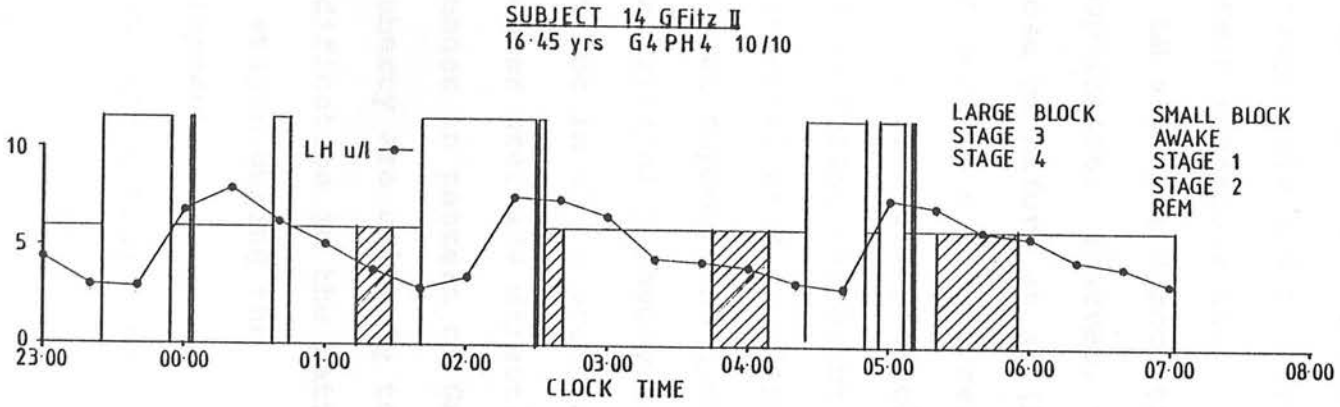


Fig 4.8

The relationship between nocturnal pulsatile LH secretion and polygraphic sleep stages. Rapid eye movement (REM) sleep is indicated by the small shaded blocks. There is no consistent pattern in the onset or cessation of LH pulses in terms of a temporal correlation with specific sleep stages in these two subjects characteristic of the whole study group.

given rise to the hypothesis that sleep, in particular slow-wave sleep, subserves a restorative role facilitating the shift in metabolic balance from degradative to synthetic processes (Adam & Oswald, 1977). LH can also be considered to be an anabolic hormone by virtue of its stimulation of gonadal steroidogenesis and the sex steroids in turn promote protein synthesis and accumulation of cyclic AMP. LH also has direct trophic effects on the Leydig cell population. However, the results in this study and those of Alford et al (1973) and Judd et al (1974) did not confirm a direct relationship between LH secretion and specific sleep stages. Furthermore, the secretion of LH during the night cannot be acutely shifted in sleep reversal studies (Kapen et al, 1974). These observations do not support the hypothesis that specific sleep stages are critically involved in anabolic metabolism, at least not in those processes under the influence of LH and sex steroids directly. It can also be said that the changes in pattern of GnRH or gonadotrophin secretion in puberty are unlikely to be a direct consequence of any modification in the pattern or content of polygraphic sleep stages during the individual's growth and development.

Over the last two decades, the pineal hormone, melatonin, has been shown to have photoperiod-induced antigonadotrophic effects in animals, especially well demonstrated in seasonal breeding species such as the hamster (Turek & Campbell, 1979). In the human, early clinical studies established the association between

tumours of the pineal gland and precocious puberty (Thamdrup, 1961). It has therefore been suggested that the pineal gland can keep puberty in check by means of inhibitory hormones. Melatonin secretion in humans is augmented during nocturnal sleep (Weinberg et al, 1979) and melatonin also induces the synchronization of EEG activity during slow-wave sleep (Anton-Tay et al, 1971). Silman et al (1979) reported in a cross-sectional study of 51 boys and girls aged 11½ to 14 years that an abrupt decline in plasma melatonin concentration occurred at the onset of puberty in boys. However, other reports were unable to confirm the presence of any meaningful changes in plasma or urinary melatonin concentration during or before puberty (Fevre et al, 1979; Lenko et al, 1981; Tetsuo et al, 1982; Ehrenkranz et al, 1982). Another pineal hormone, arginine vasotocin, a neurotransmitter peptide with higher nocturnal concentrations in the cerebrospinal fluid (Pavel, 1979) can both induce slow-wave sleep (Pavel et al, 1979) and inhibit LH secretion (Pavel & Petrescu, 1966). However, there is currently no good evidence that arginine vasotocin is directly or indirectly involved in the mechanism of puberty. Although the role of the pineal in human reproduction and puberty remains rather obscure at present, the prominent rhythmic fluctuations of pineal activity and its intimate relationship with seasonal changes in neuroendocrine-testicular functions in seasonally-breeding animals (Reiter, 1974; Licoln & Short, 1980) possess certain features analogous to the rhythmic control of nocturnal hypothalamic GnRH

secretion at the onset of puberty in humans.

The pituitary responsiveness to exogenous GnRH in puberty has been assessed by a number of investigators (Roth et al, 1972; Job et al, 1972; Kastin et al, 1972; Grumbach et al, 1974; Franchimont et al, 1975; Dickerman et al, 1976) using a single bolus injection. The later use of continuous GnRH infusion (de Lange et al, 1974; Reiter et al, 1976) has revealed the possible existence of two separate pools of LH in pubertal subjects in response to a more prolonged, continuous (Bremner & Paulsen, 1974) or intermittent GnRH stimulation (Wang et al, 1976). The first was considered to be an immediately-releasable LH pool while the second a reserve or storage pool possibly requiring protein synthesis. Using repeated pulses of GnRH at a dose and frequency (10 μg per 2 hours) that did not exceed the capacity of the pituitary gonadotropes, Yen et al (1975) were able to delineate the differential effects of gonadal steroid feedback on these two hypothetical pools of LH during the human menstrual cycle. Further support for the concept of two functional compartments in pituitary LH secretion was provided by the finding that the ratio of bio- to immuno-reactivity was greater in LH from the second compared to the first pool (Dufau et al, 1976; Beitins et al, 1977). An additional rationale for adopting the repetitive low-dose GnRH stimulation in this study is the attempt to reproduce the endogenous pattern of pituitary stimulation so that assessment of responsiveness may relate more closely to the physiological state.

The present results showed that a constant relationship between the first and second LH pools elicited by repetitive GnRH stimulation during puberty was present. They increased in parallel through the six stages of puberty with the magnitude of the second pool remaining at 60-67% of the first. There was no preferential increase in either LH pools and a priming effect on the pituitary response by the first GnRH bolus was not evident. Indeed, the subsequent responses were always smaller than the first. This pattern is most likely to be the result of the particular dose and frequency of GnRH stimulation rather than any preferential shifts of LH between the two pools or the disproportionate negative feedback of testosterone on the second LH pool. It is reasonable to conclude that the increase in gonadotrophin secretion in puberty is associated with a parallel increase in the size of the releasable and reserve LH pools in the pituitary. Recent studies in rats showed that GnRH receptors correlated closely with intrapituitary LH content and serum gonadotrophin concentration during sexual maturation in both males and females (Clayton & Catt, 1981). Thus maturational changes in pituitary GnRH receptors may mediate some of the biological action of endogenous hypothalamic GnRH secretion during puberty. It is hoped that having overcome the technical problems of studying pituitary GnRH receptor functions in vitro, further studies in this promising area may elucidate the molecular and subcellular mechanisms involved in the dynamics of

pulsatile GnRH stimulation of the pituitary.

Another way of enhancing the pituitary response is by increasing the duration of gonadotrophin release to each pulse of GnRH. Since the metabolic clearance rate of gonadotrophins does not change during sexual maturation (Ross, 1970; Licoln, 1978), any alteration in the apparent half-life of an LH secretory pulse must result from a change in the secretory dynamics of the pituitary. From the present results (Table 4.3) it is evident that the apparent half-life of each of the four consecutive LH pulses induced by exogenous GnRH remained remarkably constant throughout puberty. This reaffirms the belief that a relatively simple quantitative increase in LH reserve is characteristic of the functional maturation of the pituitary during puberty. A related finding is that the apparent half-life of the first induced LH pulse is significantly higher than the subsequent three. This suggests that the pituitary response to the first GnRH bolus is more sustained than the later ones. This may be indicative of desensitization or down-regulation of pituitary gonadotropes induced by the supra-physiological GnRH stimulation (Clayton & Catt, 1981; Nett et al, 1981).

In summary, this study examines the nocturnal episodic gonadotrophin secretory pattern and the pituitary responsiveness to repetitive GnRH stimulation during sexual maturation in a group of boys with delayed puberty. Considering that the majority of these subjects consisted of those with constitutional delayed puberty while the

few with hypogonadotrophic hypogonadism could be said to represent the prepubertal or early pubertal states, there is no reason to believe that the longitudinal or cross-sectional pattern of hormonal changes described here should not be applicable to normal puberty. It was found that (1) the earliest detectable hormonal indicator of pubertal development was the presence of nocturnal pulsatile LH secretion; (2) the pattern of nocturnal LH secretion altered quantitatively and qualitatively due to the GnRH frequency modulation of the pituitary combined later with the effects of gonadal steroid negative feedback and possibly other less well-defined neuroendocrine factors; (3) the ratio of plasma LH:FSH concentrations reversed during early puberty either as a result of the differential effects of gonadal steroidal and/or non-steroidal feedback or changes in the frequency of GnRH pulsatile stimulation; (4) the enhanced pituitary responsiveness during puberty was associated with an increase in both the releasable and reserve pools of LH; (5) the estimated magnitude of pulsatile GnRH secretion did not increase during puberty.

The relevance of these changes to the mechanism of puberty should be assessed against the background of the gonadostat and the neuroendocrine hypotheses (see Section B, Chapters 1.4.3 and 1.4.4) as well as the broader perspective of the earlier ontogenic pattern in the pre- and postnatal HPT axis. There is little doubt that sex steroid negative feedback is operative before puberty (Raimirez, 1973; Grumbach et al, 1974). There is also

ample experimental and clinical evidence that the negative feedback threshold of gonadotrophin secretion is raised from prepuberty to adulthood (Kulin & Reiter, 1972; Kelch et al, 1973; Negros-Vilar et al, 1973; Smith et al, 1977). It could therefore be postulated that the initiation of pulsatile GnRH secretion at the onset of puberty is the result of a rise in negative feedback threshold to gonadal steroids in the hypothalamic gonadostat. There is however no direct or indirect evidence from these studies to support it. Furthermore, the diphasic pattern of basal and GnRH-induced gonadotrophin secretion from infancy to adulthood is qualitatively similar in normal and agonadal individuals (Conte et al, 1975; Conte et al, 1981). The fall in GnRH/gonadotrophin secretion between 4 and 11 years in normal and agonadal children strongly suggests that intrinsic CNS inhibitory mechanism(s), independent of sex steroids, are responsible for the restraint of gonadotrophin secretion before puberty. It follows that the suppression of the neural inhibitory mechanism(s) at the onset of puberty may be responsible for the reactivation of pulsatile hypothalamic GnRH secretion irrespective of the level of gonadal steroid negative feedback. The finding in this study that pulsatile GnRH/gonadotrophin secretion, heralding the sequential maturation of the pituitary and gonads, was present only in association with nocturnal sleep in the peripubertal period favours a neural disinhibitory or excitatory mechanism in the initiation of puberty. That the same pattern of sleep-related hormonal changes are

present in patients with hypothalamic precocious puberty (Boyar et al, 1973a) and those with gonadal dysgenesis (Boyar et al, 1973) lends further weight to the neuroendocrine hypothesis.

The studies of Ojeda (1980) in rats showed that the decrease in negative feedback sensitivity became evident only after the occurrence of the first ovulation. This suggests that changes in feedback sensitivity are a consequence rather than the cause of the pubertal process. Our results are in accordance with this view. The increased frequency of hypothalamic GnRH stimulation at the onset of puberty primes the pituitary so that the basal secretion of both LH and FSH (with ratio reversed) and the LH response to GnRH became steadily enhanced even in the presence of rising gonadal steroid output. The fact that this peripubertal pattern of gonadotrophin secretion can be reproduced virtually identically in monkeys with hypothalamic lesions or in sexually infantile monkeys treated by intermittent (one hourly) GnRH administration (Knobil et al, 1980; Wildt et al, 1980) further highlights the primacy or neural mechanisms acting on the hypothalamus. In the light of these findings it seems reasonable to suggest the the original gonadostat hypothesis places an inappropriate emphasis on gonadal steroid negative feedback which we believe to be of peripheral significance only in the mechanism of puberty. The neuroendocrine control of puberty epitomizes the way in which a primarily frequency-coded neuronal signal, relayed by afferent inputs to the arcuate nucleus,

can be translated into an intermittent pulsatile hormonal message by the GnRH-secreting neurones. This study was able to demonstrate how small changes in the frequency of GnRH discharge can produce quite dramatic and acute changes in pituitary function. Thus in the temporal pattern of hormone secretion from the hypothalamus resides a novel mechanism, that of frequency-modulation, of intercellular communication which assumes major physiological importance in the initiation of puberty.

The exact mechanism by which the brain reactivates the GnRH-secreting neurones in the medial basal hypothalamus is unknown. In non-seasonal breeding species such as man, it appears that melatonin does not play an active role in the control of gonadotrophin secretion in puberty (Fevre et al, 1979; Lenko et al, 1981; Tetsuo et al, 1982; Ehrenkranz et al, 1982). It is interesting to note that CNS opiods (Morley et al, 1980; Quigley et al, 1980a; Moult et al, 1981) and dopamine (Huseman et al, 1980; Quigley et al, 1980b) may have tonic inhibitory effects on GnRH on gonadotrophin secretion. The suppression of these inhibitory influences by neuronal mechanisms in some way connected with sleep may be responsible for the initiation of puberty. This could be coupled with the concept that changes in the internal environment or body composition, when the individual reaches an appropriate stage of physical maturity, can provide the necessary signal for the brain to remove the inhibitory control on the hypothalamic "pulse generator". However, the nature of these

pulsatile metabolic signals for the initiation of puberty has so far eluded identification (Glass & Swerdloff, 1980). An alternative view derived from the study of seasonal breeding in animals is that the onset of puberty is dependent on the ability of certain areas of the brain to integrate the endogenous biological rhythms (including endocrine ones) with the 24-hour clock rhythm through sensory cues such as the light-dark or sleep-wake cycles provided by the external environment (Licoln & Short, 1980; Ramaley, 1980). Presumably, these maturational changes in the brain will depend to some extent on the formation or re-establishment of appropriate neuronal synapses at the region of the arcuate nucleus. There is some evidence from experimentally induced precocious puberty in rats that increased hypothalamic synaptogenesis may be associated with sexual maturation (Matsumoto & Arai, 1977; Arai & Matsumoto, 1978).

CHAPTER 5

THE GONADAL RESPONSE IN PUBERTY

5.1 Introduction

The gonads play an active part in sexual maturation as suggested by the enhanced testicular responsiveness to hCG stimulation (Winter et al, 1972; Schöller et al, 1975). The mechanism for this gonadal amplification of the primary neuroendocrine signal for sexual development is still unclear in spite of an increasing body of animal experimental data on the regulation of Leydig cell functions (for review see Purvis & Hansson, 1978; Bartke, 1978; Catt et al, 1980; Swerdloff & Heber, 1981; Risbridger et al, 1981; Sharpe, 1982). From these studies, it is becoming clear that not only LH, which is traditionally the primary trophic hormone, but also FSH and prolactin may be necessary for the development and maintenance of adult Leydig cell functions in some lower animals (rodents mainly). In humans, the situation is complicated by the presence of circadian and circhoral variations in several of the relevant pituitary and gonadal hormones which first become manifest at this stage of development. Hence prior to extrapolating some of the newly-acquired understanding in testicular physiology to human puberty, it is necessary to examine in some detail the temporal relationships between observed changes in testicular steroidogenic response and the putative trophic or regulatory hormones - LH, FSH and

prolactin, taking into account both the episodic and the sleep-related secretory patterns of these hormones. A further object of this study is to consider the role of LH in the development of Leydig cell function in puberty in terms of the pulsatile or intermittent nature of the trophic stimulus.

5.2 Results

A significant linear rising trend ($p<0.001$) in mean testosterone concentration in the evening (20.00-23.00 h), night (23.00-07.00 h) and morning (07.00-09.00 h) was present across the six stages of puberty (Fig 5.1). Adult levels of testosterone (>350 ng/100 ml) were reached around stage 3 to 4 in the morning and a little later during the night. The time at which maximal rate of increment and peak concentrations of testosterone were achieved lagged behind that for LH by one or two clinical stages (Fig 5.1). In the later stages of puberty, when no further increase in mean LH concentrations occurred, testosterone nonetheless continued to rise. However, a significant nocturnal elevation in mean testosterone concentration was first detectable in stage 1B, the time when sleep-related LH secretion also became established (Fig 5.2). The nocturnal rise in testosterone concentrations continued throughout puberty and indeed increased in magnitude with maturity so that the largest increments were observed in stages 4 and 5 (Fig 5.2). Although LH levels fell abruptly on waking in the morning, mean testosterone concentrations showed no

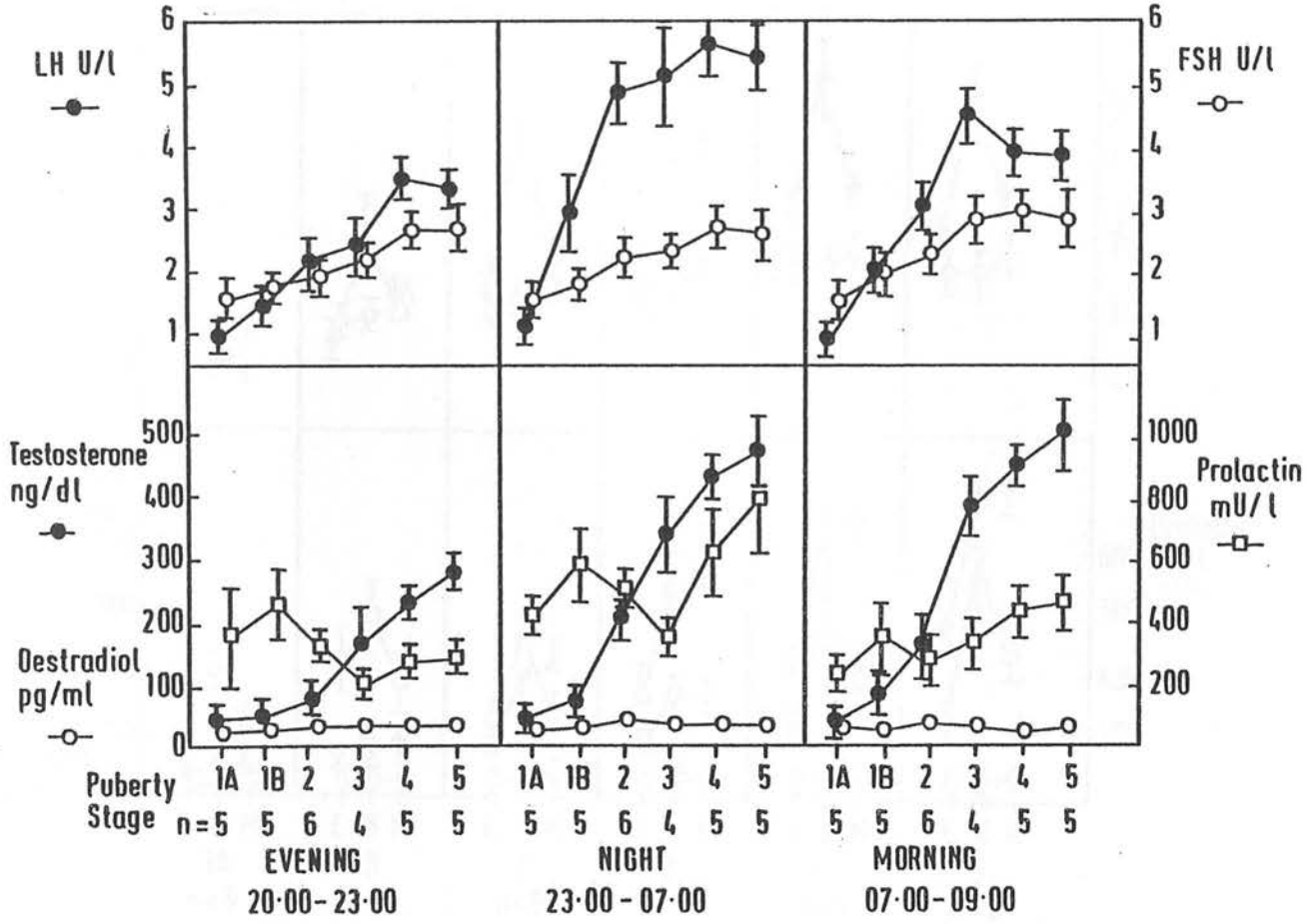


Fig 5.1

Mean (SEM) plasma LH, FSH, prolactin, testosterone and oestradiol in the six stages of puberty (see Chapter 3) at three different parts of the 24-hour cycle: Evening (E) 20.00-23.00 h, night (N) 23.00-07.00 h and morning (M) 07.00-09.00 h.

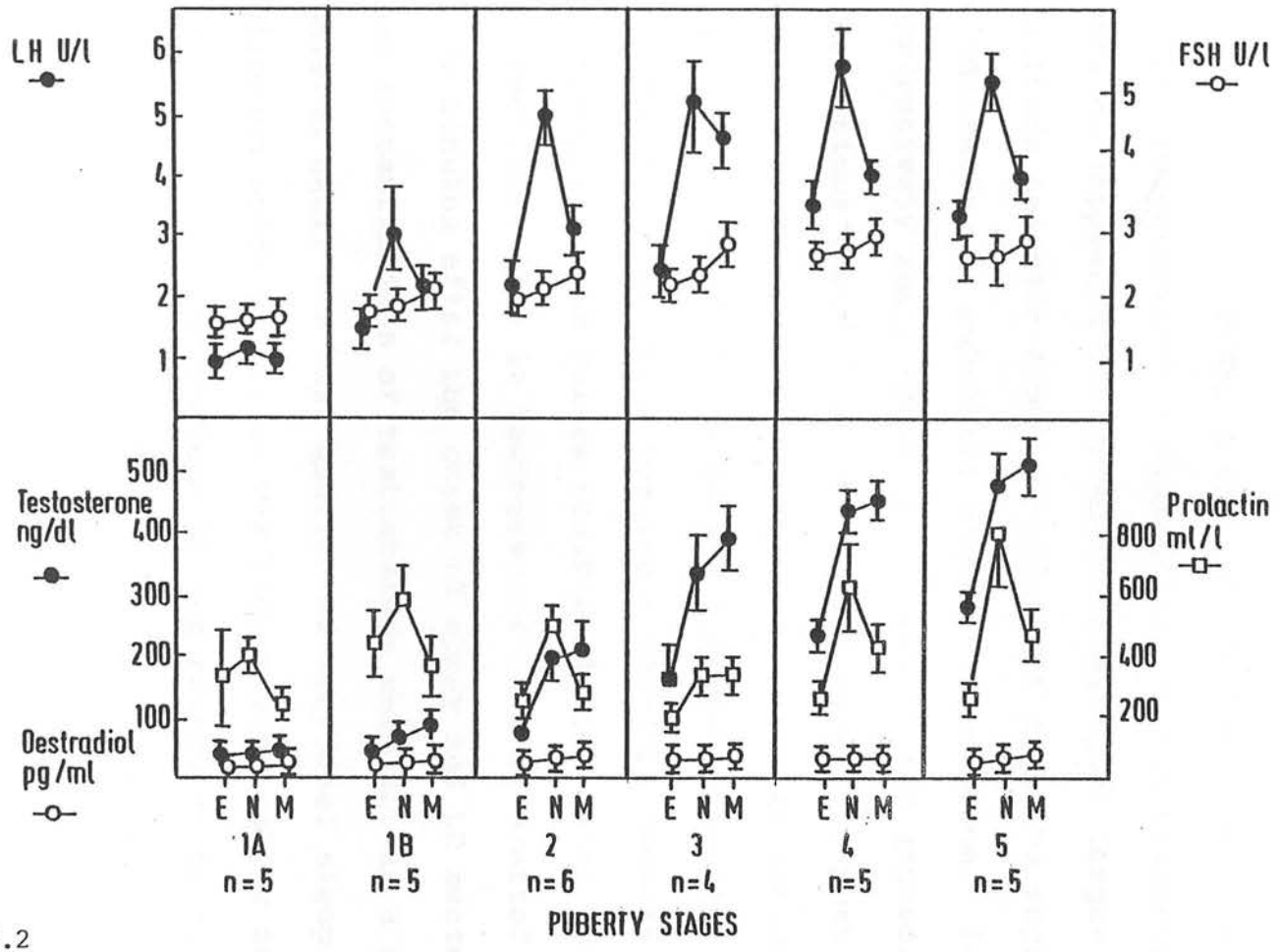


Fig 5.2

The diurnal pattern of mean (SEM) plasma LH, FSH, prolactin, testosterone and oestradiol in the 6 stages of puberty (see Chapter 3). Evening (E) 20.00-23.00 h, night (N) 23.00-07.00 h, and morning (M) 07.00-09.00 h.

significant decline (Fig 5.2). Mean LH concentration correlated well with mean testosterone levels during puberty in the evening, night and morning (Kendall's rank correlation 0.62, 0.60 and 0.57 respectively). Mean FSH concentration correlated less well with testosterone than LH in all three parts of the 24-hour cycle (Kendall's rank correlation 0.39, 0.30, 0.33 for evening, night and morning respectively). Examination of the testosterone profiles (Appendix II) revealed no distinct large-amplitude episodic fluctuations which could be regarded as indicative of pulsatile glandular secretion. Instead, comparatively small amplitude irregular and unpredictable fluctuations (mean CV 21.3% ± 12.7) were seen both asleep and awake. There were however occasional exceptions, for example subject GFII, in whom a distinct pulsatile pattern of testosterone bearing a constant temporal relationship to LH pulses could be demonstrated (Fig 5.3). The nocturnal rise in testosterone usually started some 60-90 minutes after the onset of sleep and LH secretion. Peak concentrations of testosterone were not as a rule achieved until the last quarter of nocturnal sleep following which a decline was observed on waking in the morning. This hour-to-hour LH and testosterone relationship demonstrating the phase-delayed testicular response to nocturnal pituitary LH secretion was only apparent on scanning individual profiles (Appendix II). Thus although the mean morning and nocturnal testosterone concentrations were not significantly different at any stage of puberty, the profiles consistently demonstrated the declining

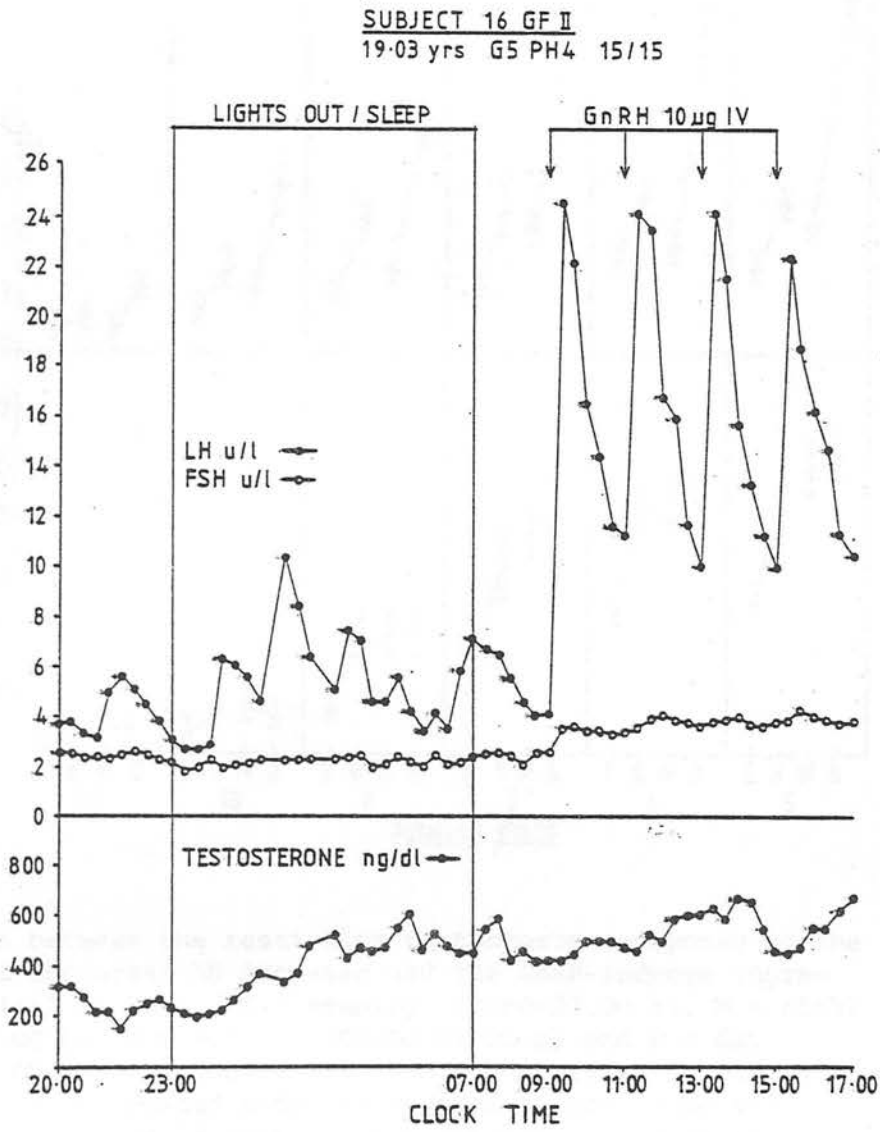


Fig 5.3

Plasma LH, FSH and testosterone concentrations between 20.00-17.00 h in study II of subect 16 GF. Unlike the majority, pulsatile fluctuations of testosterone concentration were discernible in this subject. The testosterone peaks bore a constant temporal relationship to LH, lagging behind the endogenous nocturnal LH pulses by 20-40 minutes. The relationship between the GnRH-induced LH peaks and the fluctuating testosterone concentration was less clear.

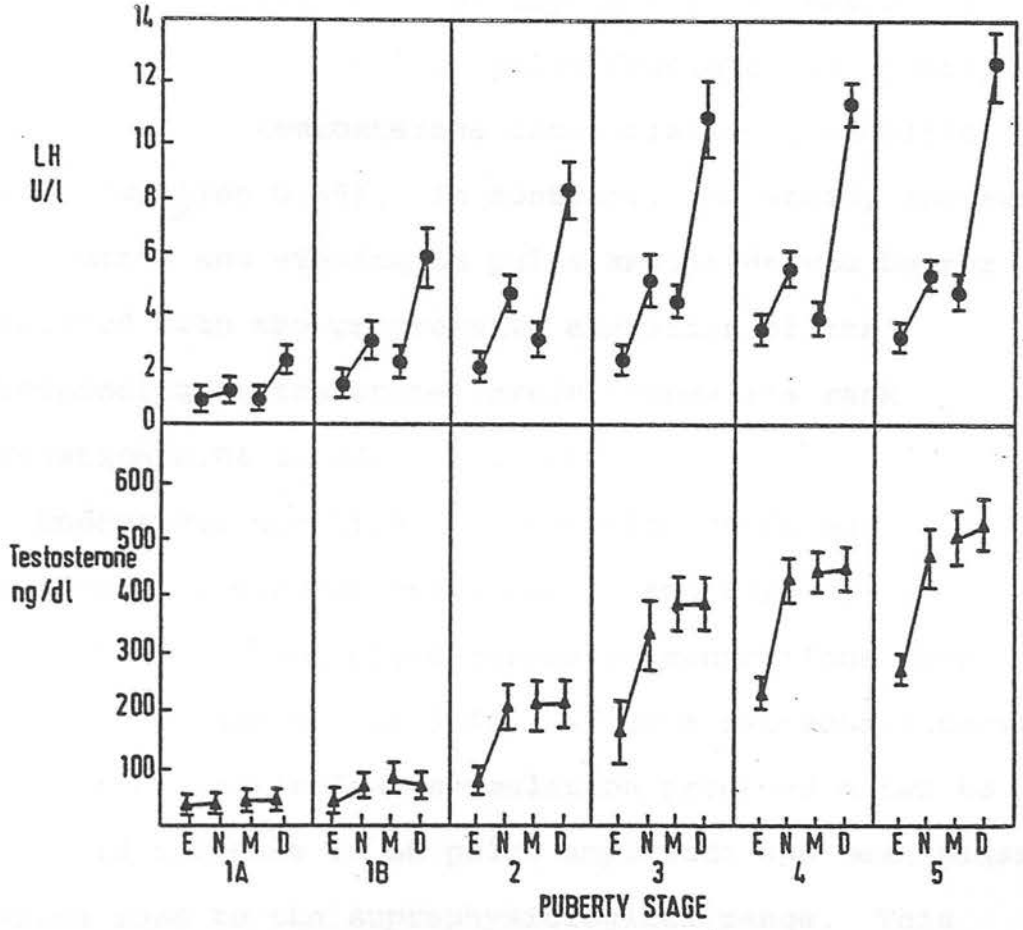


Fig 5.4

Comparison between the testicular testosterone response to the endogenous nocturnal LH increase and the GnRH-induced supra-physiological LH rise. E = evening (20.00-23.00 h), N = night (23.00-07.00 h), M = morning (07.00-09.00 h) and D = day (09.00-17.00 h). A significant ($P<0.05$) increase in plasma testosterone associated with the nocturnal rise in LH was observed from stage 1B onwards. In contrast, despite the greater magnitude of LH increase induced by exogenous GnRH, there was no significant difference between the morning (M) and daytime (D) concentrations of testosterone at any stage of puberty.

levels of testosterone after waking.

When nocturnal LH was considered in terms of pulse frequency and amplitude, it was evident that the most rapid increases in pulse frequency in stages 1A to 2 were associated with minimal Leydig cell secretion of testosterone. Nocturnal LH pulse frequency was poorly correlated with testosterone concentration (Kendall's rank correlation 0.36). In contrast, the steady increase in nocturnal and evening LH pulse amplitude was better correlated with the progressive elevation of the corresponding testosterone levels (Kendall's rank correlation 0.64 in each instance).

Endogenous nocturnal LH secretion produced significant testicular responses in the form of elevations in plasma testosterone concentrations from stage 1B to stage 5 (Fig 5.4). After a two-hour interval, exogenous pulsatile GnRH stimulation produced a two to three fold increase in LH pulse amplitude and mean plasma LH which rose to the supraphysiological range. This however did not produce a further increase in the plasma testosterone concentrations from those in the morning (Fig 5.4). The testosterone profiles demonstrated that, during this eight hour period of exogenous GnRH stimulation, the declining trend normally present after waking was reversed and the relatively high testosterone levels of the early morning was maintained for the duration of this spell.

Oestradiol levels remained low (20-35 pg/ml) during puberty with no obvious diurnal variation (Fig 5.2). No

significant change in mean evening or night oestradiol concentrations was observed across the pubertal stages. A significant stage effect was however found in the mean morning oestradiol with concentrations rising to 33.1 ± 8 and 31.5 ± 6 pg/ml in stages 2 and 3 respectively (Figs 5.1 & 5.2). This early to mid pubertal oestradiol increase was not sustained towards the second half of puberty when oestradiol levels were not significantly different from stage 1A and 1B.

Results of mean prolactin concentrations in the evening, night and morning during puberty are presented in Figures 5.1 and 5.2. Nocturnal rise of prolactin was observed in all stages of puberty although this only reached the 5% level of statistical significance from stage 2 onwards. The mean evening and nocturnal prolactin concentrations did not show any significant change during puberty. A small but significant ($p<0.05$) linear trend of increasing prolactin concentration in the morning was present. Individual prolactin profiles demonstrated the enhanced episodic secretion during nocturnal sleep (Fig 5.5). Although three to four secretory episodes were observed in most patients during the eight hours of sleep, there was no consistent pattern in nocturnal prolactin secretion during puberty.

5.3 Discussion

In this section, the control of gonadal development in human male puberty was studied. The use of multiple day-and-night blood sampling has made it possible to

Fig 5.5

Plasma prolactin profiles in two subjects DF and ST studied on three and four occasions respectively as they progressed through puberty. The nocturnal rise in prolactin with three to four major secretory episodes was observed in all stages of puberty. There was no apparent trend in the pattern or magnitude of prolactin secretion during pubertal development.

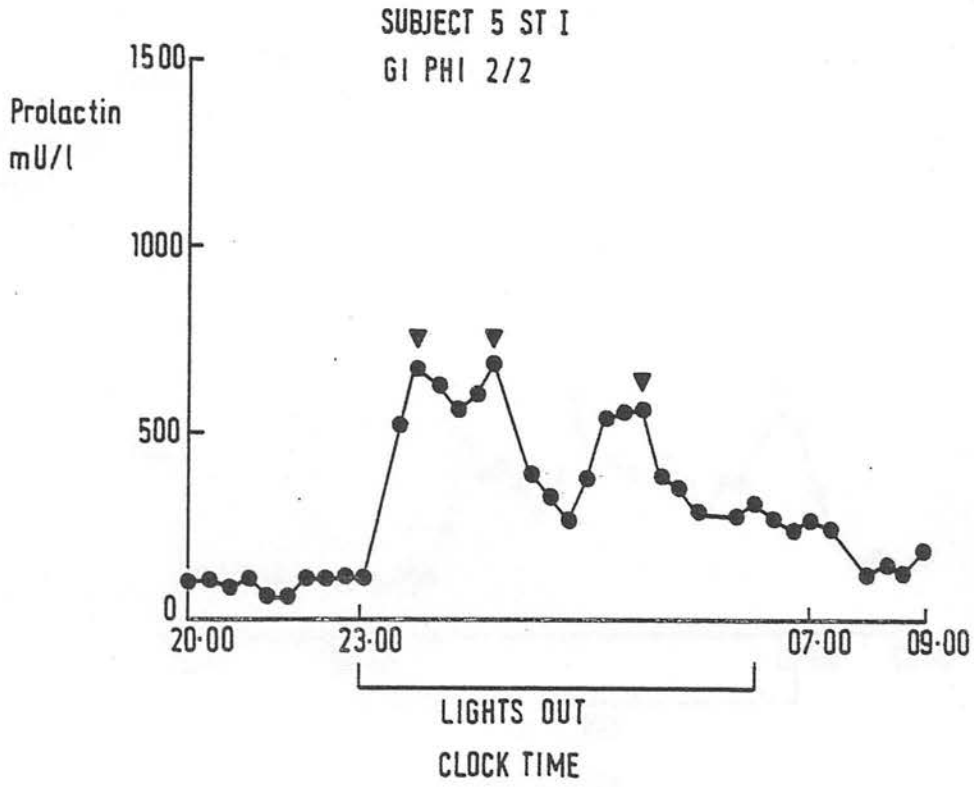

SUBJECT 5 ST I
GI PHI 2/2
Prolactin
mU/l
1500
1000
500
0
20·00
23·00
07·00
09·00
LIGHTS OUT
CLOCK TIME

Fig 5.5

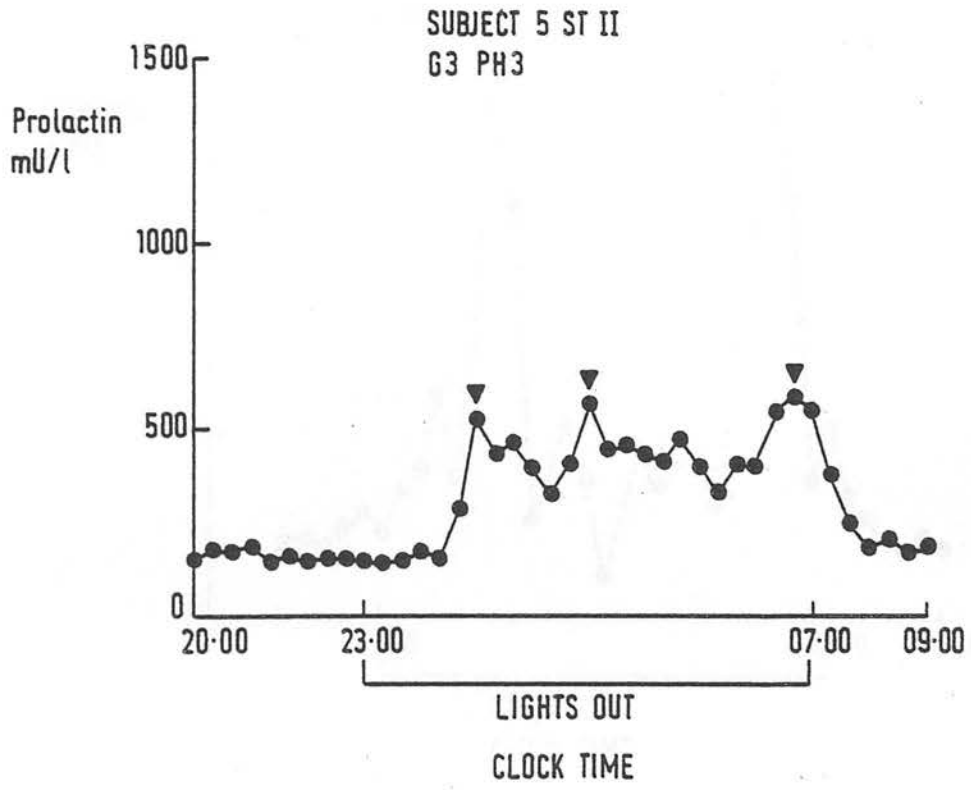

SUBJECT 5 ST II
G3 PH3
Prolactin
mU/l
1500
1000
500
0
20·00
23·00
07·00
09·00
LIGHTS OUT
CLOCK TIME

Fig 5.5

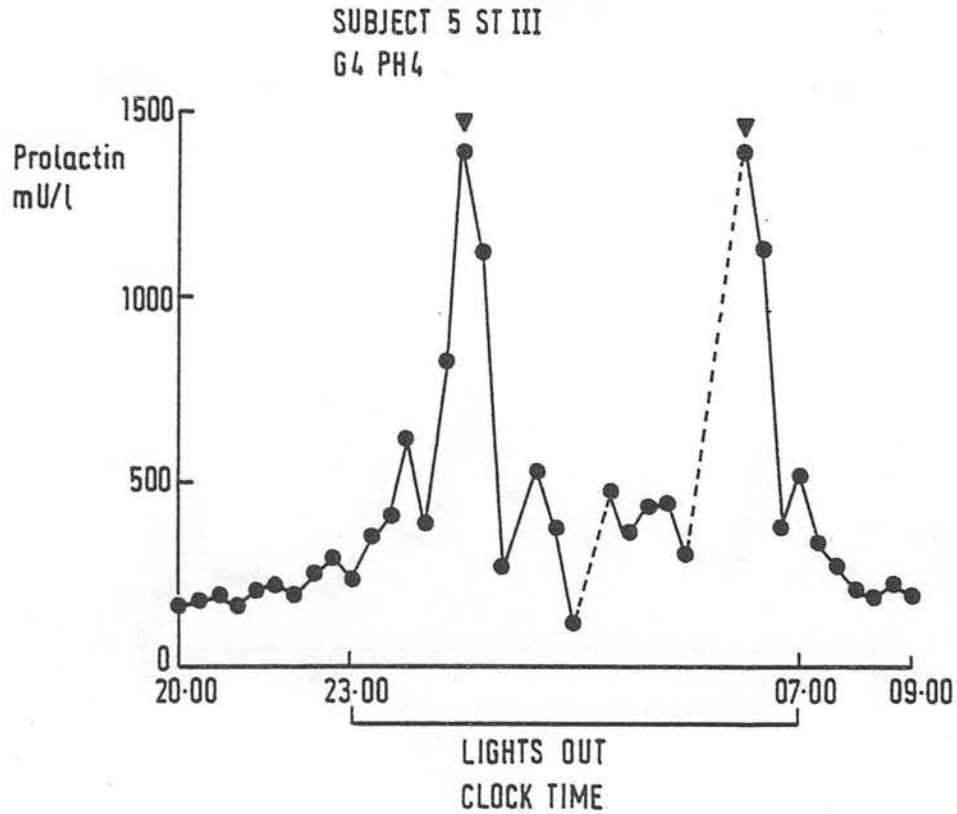

SUBJECT 5 ST III
G4 PH4
Prolactin
mU/l
1500
1000
500
0
20·00
23·00
07·00
09·00
LIGHTS OUT
CLOCK TIME

Fig 5.5

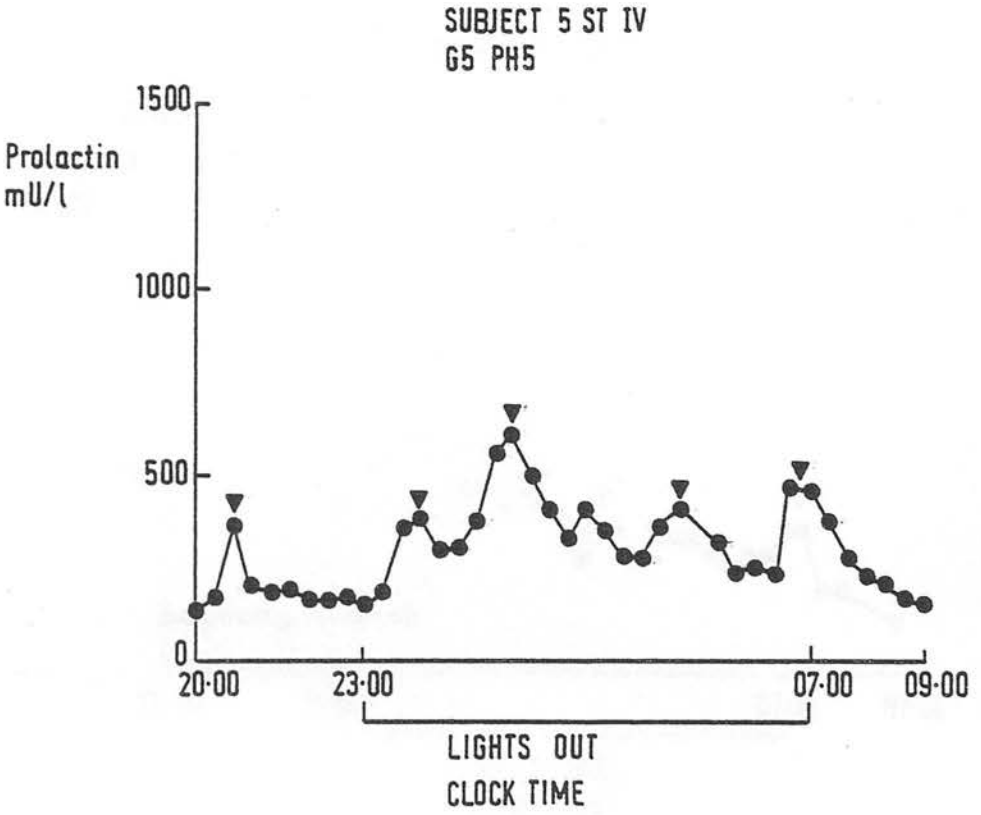

SUBJECT 5 ST IV
G5 PH5
Prolactin
mU/l
1500
1000
500
0
20·00
23·00
07·00
09·00
LIGHTS OUT
CLOCK TIME

Fig 5.5

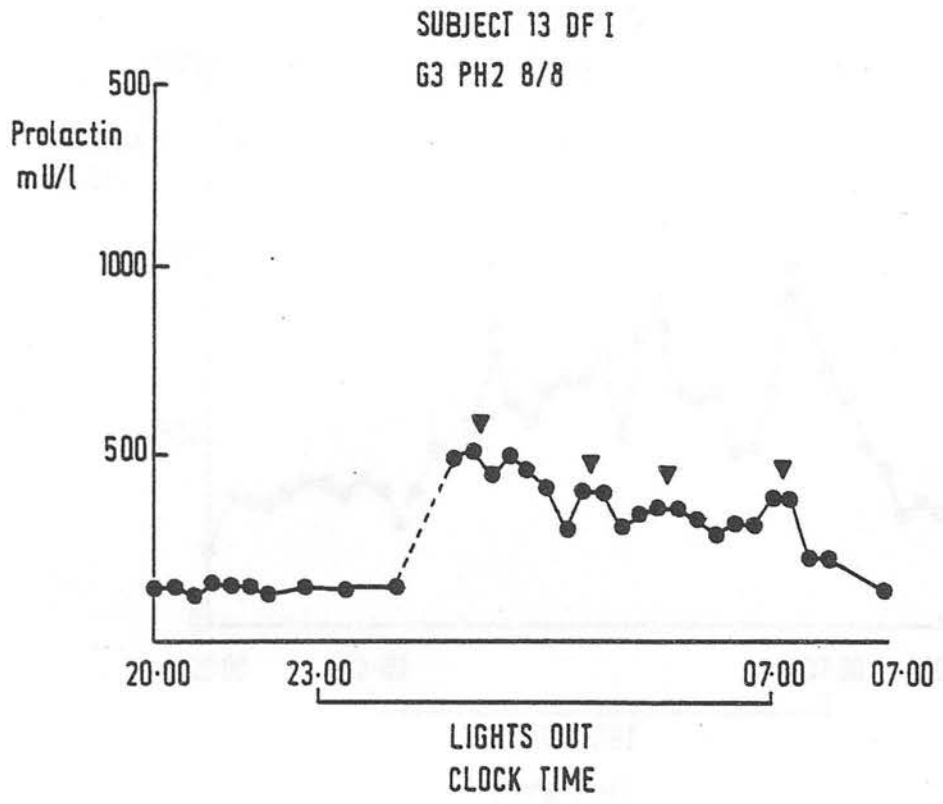

SUBJECT 13 DF I
G3 PH2 8/8
500
Prolactin
mU/l
1000
500
20·00
23·00
07·00
07·00
LIGHTS OUT
CLOCK TIME

Fig 5.5

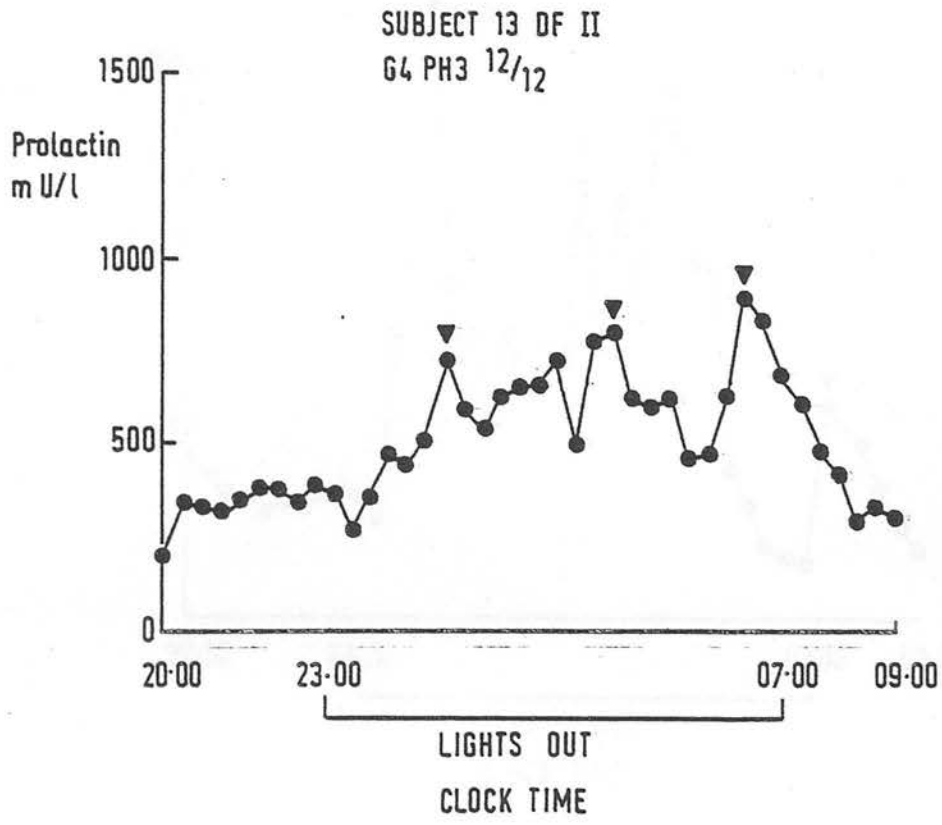

SUBJECT 13 DF II
G4 PH3 12/12
1500
Prolactin
m U/l
1000
500
0
20·00
23·00
07·00
09·00
LIGHTS OUT
CLOCK TIME

Fig 5.5

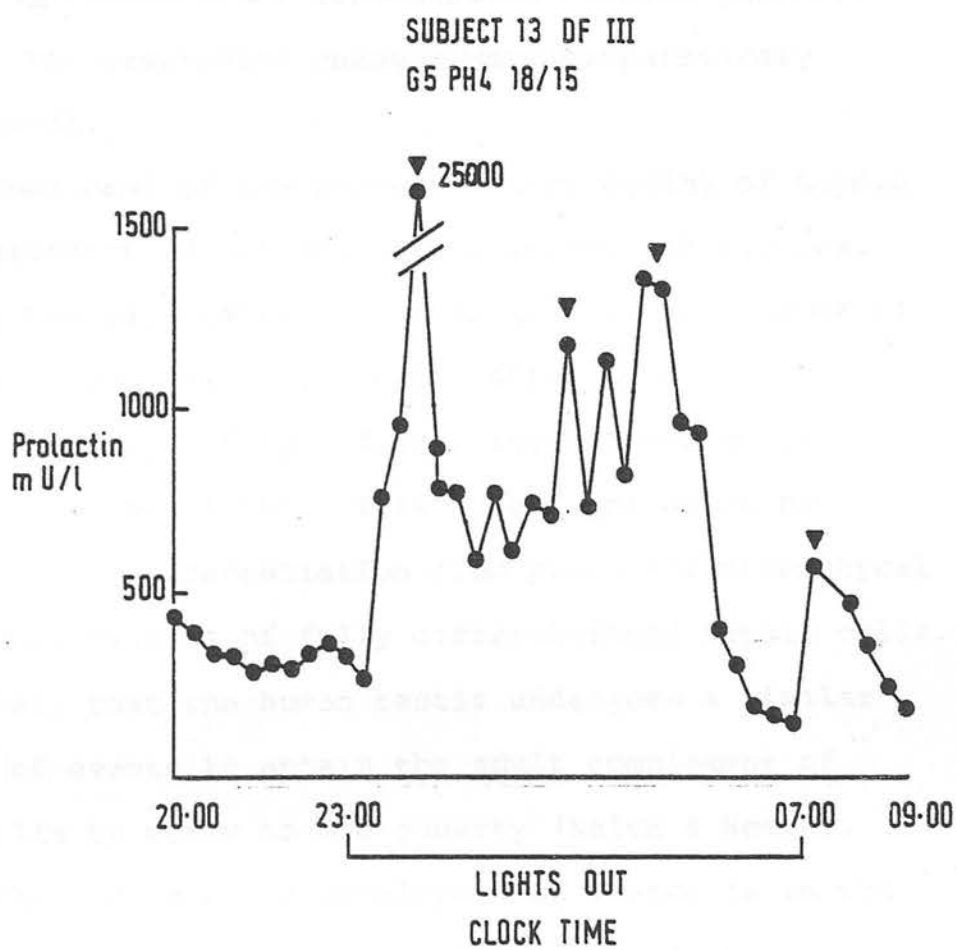

SUBJECT 13 DF III
G5 PH4 18/15
25000
1500
1000
500
Prolactin
m U/l
20·00
23·00
07·00
09·00
LIGHTS OUT
CLOCK TIME

Fig 5.5

examine the circadian and circhoral relationships between gonadal steroids and pituitary hormones. The significant correlation between mean plasma LH and testosterone at different parts of the 24 hour cycle during gonadal development is compatible with a prime trophic role for this gonadotrophin. The FSH pattern was temporally less well correlated to Leydig cell function as indicated by circulating testosterone concentration during puberty. Prolactin and oestradiol changes were comparatively insignificant.

A great deal of the present understanding of Leydig cell development is derived from experimental studies, mostly on the rat testis. In this animal, the number of Leydig cells per testis increases three to ten fold between 36-60 days of age (Clegg, 1966; Knorr et al, 1970; Pahne et al, 1975). This is brought about by a combination of differentiation from precursor mesenchymal cells and by mitosis of fully differentiated Leydig cells. It is likely that the human testis undergoes a similar sequence of events to attain the adult complement of Leydig cells by early to mid puberty (Kaler & Neaves, 1978). The second major developmental change is in the number of testicular LH receptors which increase about twenty fold from 14-70 days of age in the maturing rat (Pahne et al, 1975; Ketelslegers et al, 1978). Even though most of this increase in receptor numbers reflects the expanding Leydig cell population, there is some evidence to support an increase in number of LH receptors per cell (Thanki & Steinberger, 1976). That the

development and maintenance of the testicular LH receptor population is under gonadotrophin control is suggested by the finding that hypophysectomy results in a dramatic fall in LH receptor numbers (Frowein & Engle, 1975; Thanki & Steinberger, 1976). No comparable data in the human testis exist at present. The testicular steroidogenic pathway provides the third mechanism for gonadal activation during puberty. The steroidogenic response to LH stimulation in adult rats is five to six times higher than that in immature animals (Odell et al, 1974). With the exception of 17β-hydroxysteroid dehydrogenase, activities of all other enzymes in the biosynthetic pathways of testosterone are at a maximum in the Leydig cells of the rat by 30-35 days of age (Inano et al, 1967; Payne et al, 1977). Five alpha-reductase activity is high in the prepubertal rat testes so that the major androgens produced at this time are 5α-reduced metabolites of testosterone such as 3α-Diol and androsterone (Moger, 1977 & 1979; Foldesy & Leatham, 1980; Purvis et al, 1980). The gradual fall in 5α-reductase activity with increasing maturity is responsible for the shift in testicular androgen synthesis and secretion in favour of testosterone compared to 5α-reduced androgens. Thus, despite the fifteen to twenty fold increase in testosterone production with maturity, the total production of 17β-hydroxy androgens (testosterone, 5α-dihydrotestosterone, 3α-diol, 3β-diol and androsterone) per Leydig cell may actually decrease during sexual development. In humans, the principle androgen in

prepubertal subjects is androstenedione which increases only minimally during puberty compared with testosterone (Frasier & Horton, 1966). In vitro studies in testicular biopsy tissues from two prepubertal hypogonadotrophic patients demonstrated increased conversion of precursors to 5α-reduced metabolites (Steinberger et al, 1974a). It has also been postulated that decreased activity of 20α-hydroxysteroid dehydrogenase at puberty reduces the competitive inhibition of 17α-hydroxylation of pregnenolone and progesterone (Fan et al, 1974). It seems therefore that in humans, as in lower animals, qualitative changes in testicular steroidogenesis during puberty are as important as quantitative changes.

Luteinizing hormone has well-recognized trophic effects on Leydig cells inducing both hypertrophy (de Kretser et al, 1967; Heller & Leach, 1971) and proliferation in both immature (Chemes et al, 1976) and adult (Christensen & Peacock, 1980) rats. In immature animals, the early effect of hCG was to stimulate Leydig cell mitosis subsequently followed by increased differentiation of precursor to Leydig cells (Chemes et al, 1976). The progressive fall in Leydig cell responsiveness in adult hypophysectomized rats can be prevented by LH administration (El Safoury & Bartke, 1970; Van Beurden et al, 1976; Zipf et al, 1978). However, maintenance of steroidogenic activities in Leydig cells may merely reflect the effect of chronic LH/hCG treatment on Leydig cell hypertrophy or hyperplasia. Whether these non-physiological changes can be

related to functional alterations of the qualitative or quantitative aspects of steroidogenesis as discussed above is yet to be established.

Results in this study indicated that LH plays a dominant role in the development of enhanced testicular responsiveness during puberty in humans. LH changes paralleled but preceded that of testosterone and the nocturnal rise in circulating testosterone in early and mid puberty always followed a significant increase in LH secretion. It may be speculated that the nocturnal high frequency small amplitude LH stimulation, which is characteristic of early puberty and gives rise to only minimal increase in mean testosterone levels, is important in stimulating Leydig cell differentation and mitosis and increasing the number of LH receptors per Leydig cell. As puberty advances, the increasing amplitude of LH secretion may induce the various shifts in the pattern or enhanced rates of steroidogenesis in the full complement of Leydig cells producing the rapidly increasing testosterone response seen in later stages. Thus an appropriately high frequency of LH pulses may be the crucial factor governing the increase in Leydig cell numbers and LH receptors but given a stable pulse frequency of once every two hours, it is the amplitude changes which modify the steroidogenic pattern. Obviously, much more work is needed before these postulates can be verified. A similar priming effect on Leydig cells by repeated small doses of LH has been demonstrated in the juvenile monkey (Arslan et al, 1978).

There is also evidence that repeated small priming doses of LH are responsible for the acquisition of LH responsiveness during early sexual development of the rat (Purvis et al, 1979).

The physiological regulation of Leydig cell function by LH normally operates through the activation and utilization of a minute fraction (<1%) of the total receptor population (Catt et al, 1978). The large population of spare receptors may serve to maintain optimal sensitivity of the Leydig cells to hormone stimulation. When the Leydig cells are exposed to high sustained concentrations of endogenous or exogenous LH, receptor loss by internalization is induced (down regulation)(Hsueh et al, 1976; Sharpe et al, 1976; Catt et al, 1979). When receptor loss exceeds replenishment, a net fall in receptor numbers occurs with the resultant fall in sensitivity to LH stimulation. The priming effect of repeated small doses as opposed to sustained high doses of LH may serve to increase the steroidogenic capacity of Leydig cells and enhance or maintain their responsiveness to LH without incurring a degree of receptor loss that negates against these effects. The pulsatile stimulation by physiological concentrations of LH intermittently erodes a tiny percentage of the total receptor population which can be replenished before the next LH stimulus. It is conceivable that not only the maintenance but also the differentiation of adult responsiveness in Leydig cells is governed by similar principles.

In pubertal boys, as in adults, episodic changes in plasma concentrations of testosterone are not clearly recognizable as discrete secretory pulses. This contrasts with the distinct pulsatile pattern of LH to which plasma testosterone fluctuations bear a relatively loose temporal relationship. Unlike humans, pulsatile variations in plasma LH and testosterone are tightly coupled in lower species (rat - Desjardins, 1981; ram - Sandford et al, 1974; monkey - Plant et al, 1981). This apparent discrepancy may be attributed to the sluggish Leydig cell response in man to LH stimulation so that an LH secretory pulse is followed by a detectable rise in plasma testosterone only after an interval of 20-180 minutes (Naftolin et al, 1973; Judd et al, 1974b; Rowe et al, 1975; de Kretser et al, 1977). A similar phase-delayed (60-90 minutes) testosterone response induced by LH secretion associated with sleep was observed in the present and previous studies (Boyar et al, 1974a; Judd et al, 1974a). Several factors may contribute to this phenomenon. The presence of sex hormone binding globulin greatly increases the half-life of circulating testosterone in humans and provides a large capacity reservoir which dampens down any rapid fluctuations (Baird et al, 1969; Anderson, 1974). However, the presence of sex hormone binding globulin in the ram (which shows well defined pulsatile testosterone secretion), albeit of lower affinity than in man, argues against this as the sole explanation. The Leydig cell response to LH may be genuinely delayed, reflecting

perhaps the relatively high intratesticular and circulating testicular concentrations in man compared to other species (de Kretser et al, 1977; Serio et al, 1980). Thus if the Leydig cells are already functioning at near maximal capacity under basal conditions, it may not be possible for the testis to respond to further LH stimulation by acute increases in pulsatile testosterone secretion of large amplitudes. A third possible explanation may be provided by the finding that a reduction in the LH pulse frequency by a synthetic androgen resulted in a closer temporal relationship between plasma LH and testosterone concentrations (Vigersky et al, 1976). The adjacent LH pulses may be so closely spaced that the relationship between LH and testosterone is obscured. Further work in this area is required before any one of these possible explanations can be substantiated.

Nocturnal plasma testosterone steadily increased towards peak levels before waking in response to the endogenous sleep related pulsatile LH secretion. Raising the LH concentration further, even at the presumed optimal pulse frequency (2 hourly), by GnRH stimulation failed to increase the testosterone concentrations beyond the maximal concentrations found in the early morning. This implies that at each stage of pubertal development, Leydig cell steroidogenesis operates at near maximal capacity. This interpretation is compatible with the observation that intratesticular and circulating testosterone concentrations in man are relatively high

compared to other species, as mentioned earlier. That the Leydig cells are not refractory to LH stimulation in the morning is suggested by two observations. Firstly, testosterone concentrations normally decline steadily from the early morning peak to a nadir some time in late evening (Boyar et al, 1974a; Sjöberg et al, 1979). The explanation of this diurnal variation in plasma testosterone concentration is not clear. Secondly, in all cases in the present study, this decline in testosterone concentration in the morning was reversed shortly after the start of exogenous GnRH stimulation so that maximal concentrations were maintained through the day by the supraphysiological LH stimulus. These findings suggest that the human Leydig cells, from an early stage of development, function normally with little additional steroidogenic reserve and/or that negative regulatory mechanisms come into play as soon as the physiological range of stimulus is exceeded. The finding that pharmacological doses of hCG or LH produced a biphasic rise of testosterone levels in man (Forest et al, 1979; Wang et al, 1980) does not detract from this interpretation since the testicular response in those situations is mainly dependent on the trophic effects on Leydig cells rather than a direct stimulation of steroidogenesis. Irrespective of whether LH levels are pharmacological or supraphysiological, it is likely that significant loss in testicular LH receptors accompanied by the desensitization of steroidogenesis would have been induced in each instance (Hsueh et al, 1976; Sharpe, 1976;

Zipf et al, 1978). Furthermore, GnRH may have direct negative effects on Leydig cell LH receptors and steroidogenesis (Hsueh & Erikson, 1979; Bambino et al, 1980; Sharpe, 1981). These negative regulatory mechanisms may prevent the Leydig cells from being over-stimulated and imposes a ceiling to their responsiveness.

Luteinizing hormone rise during puberty in the rat is comparatively small and correlates rather poorly with morphological changes in Leydig cells (Swerdloff et al, 1971; de Jong & Sharpe, 1977; Payne et al, 1977; Ketelslegers et al, 1978). FSH on the other hand rises early in puberty in many species (rat - Swerdloff et al, 1971; de Jong & Sharpe, 1977; ram - Lee et al, 1976; human - Faiman & Winter, 1974; Grumbach et al, 1974) showing a striking correlation with the increase in Leydig cell population in puberty (van der Molen & Rommerts, 1981). Although Leydig cell responsiveness in adult hypophysectomized rats can be maintained by LH alone, FSH as well as LH are required in immature hypophysectomized animals (El Safoury & Bartke, 1974; Van Beurden et al, 1976). There is evidence showing that FSH can increase the number of LH receptors in the immature rat testis (Odell & Swerdloff, 1976; Chen et al, 1976 & 1977). In cryptorchid boys and hypogonadotrophic subjects, basal testosterone and the response to hCG were correlated with FSH but not LH (Sizonenko et al, 1977). This has been interpreted as evidence that FSH is important in the control of Leydig cell function in the immature human testis. FSH levels also paralleled the

increase in 17β-hydroxysteroid dehydrogenase activity in the maturing rat and is also able to stimulate the activity of the enzyme in the hypophysectomized immature rat (Payne et al, 1977; Murono & Payne, 1979). It is interesting to note that the trophic effect of FSH has been demonstrated exclusively in the immature testis. Taken together, these studies present an impressive body of evidence, albeit indirect, supporting a role for FSH in the control of the development of testicular responsiveness in the immature rat. One reservation against the full acceptance of this view is the fact that FSH binds only to Sertoli cells and some germ cells in the testis (Orth & Christensen, 1977). Thus any effect of FSH on Leydig cells is likely to be mediated via the Sertoli cells. It is possible that the Sertoli cells, when stimulated by FSH, produce local testicular factor(s) that exert trophic effects on Leydig cells (Risbridger et al, 1981).

The present study showed that FSH concentrations were higher than those of LH at the onset of puberty and that FSH gradually increased during puberty although the mean concentrations were poorly correlated with testosterone. These data are compatible with the concept that FSH may have a synergistic role in the early stages of puberty in the differentiation of Leydig cells and their LH receptor population. It is less likely, although not completely excluded, that FSH plays a significant part in the subsequent development of Leydig cell steroidogenesis. The role of FSH on spermatogenesis and seminiferous

tubular development has been discussed elsewhere (Chapter 3). Immature rat Sertoli cells in vitro can synthesize oestradiol in the presence of FSH and testosterone (Dorrington & Armstrong, 1975). In the pubertal animal, increasing circulating and intratesticular concentrations of FSH and testosterone may provide the appropriate stimulus to the developing Sertoli cells for oestrogen synthesis. It is therefore interesting to note that in this and previous studies (vide infra) a small but definite increase in oestradiol and oestrone plasma concentrations has been found. The consequence of increased intratesticular oestrogen in the developing testis is unknown but the rising circulating oestradiol level may be expected to have distant effects such as gynaecomastia (Large et al, 1980) or raised pituitary prolactin secretion.

Prolactin differs from pituitary LH and FSH in many aspects of its control and functions. The two relevant features in the context of testicular development are (1) that prolactin by itself does not have any known biological action on the testes and (2) that increased levels of this hormone are associated with enhanced Leydig cell function in some species, e.g. rat and hamster (Bartke, 1978), but regressed function in others, e.g. ram (Licoln & Short, 1980).

In the immature rat, plasma prolactin rose between 30 to 55 days of age (Negro-Vilar et al, 1973; Dohler & Wuttke, 1975) in parallel with the increase in circulating testosterone and expanding number of

testicular LH receptors. Further, the effects of prolactin in the hereditary dwarf mice or hypophysectomized rats on testicular functions was not unlike that seen in sexual maturation (Bartke, 1978). These observations formed the basis for the belief that prolactin, in conjunction with LH and FSH, is required to effect the optimal gonadal developmental changes during puberty in rodents. In human puberty, published reports are in agreement that prolactin levels do not increase in the male (Guyda & Frieson, 1973; Lee et al, 1974; Ehara et al, 1975; Aubert et al, 1977; Thorner et al, 1977). None of these studies however took note of the episodic secretion of prolactin and the increased rate of secretion during nocturnal sleep (Sassin et al, 1973; Parker et al, 1973). More recently, the nocturnal sleep-related prolactin secretion in prepubertal and pubertal children has been confirmed (Finkelstein et al, 1978; Warne et al, 1979; Large et al, 1980; Beck & Wuttke, 1980). There was no difference in the diurnal pattern of pulsatile secretion.

The present study provided further information on the diurnal secretory pattern of prolactin in subjects representing all stages of pubertal development. Longitudinal data from follow-up studies were available from nine out of sixteen boys. The results confirmed the enhanced prolactin secretion during nocturnal sleep. However the most striking finding was the tremendous variability in the timing and magnitude of prolactin secretory episodes during sleep such that no progressive

patterns were discernable during pubertal maturation even within individual subjects studied repeatedly. This is in agreement with the findings in previous studies (Finkelstein et al, 1978; Warne et al, 1979; Large et al, 1980; Beck & Wuttke, 1980). Surprisingly, mean prolactin concentration on waking in the morning (07.00-09.00 h) did show a small but significant ($p<0.05$) rising trend during pubertal maturation. The interpretation of this finding is rather difficult considering the lack of similar trends at other parts of the 24 hour cycle and the insignificant correlation between mean prolactin and oestradiol concentrations found in this and other studies (Thorner et al, 1977; Large et al, 1980) in pubertal boys.

It is interesting that the mean oestradiol concentration in the morning did increase in this study but the rise showed a different pattern to that of prolactin being significantly higher in stages 2 and 3 only. This rather peculiar pattern in plasma oestradiol in the morning has not been observed in previous studies (Bidlingmaier et al, 1973; Angsusingha et al, 1974; Gupta et al, 1975; Lee & Migeon, 1975; Baker et al, 1976; Thorner et al, 1977) which demonstrated a small but significant progressive increase in oestradiol concentrations between stage 2 and stage 5 of puberty. The source of circulating oestradiol in children and adolescents has not been studied although it is well established in adults that a quarter of oestradiol blood production is derived from testicular secretion while the remainder is formed by peripheral conversion from

testosterone (Longcope et al, 1969; Kelch et al, 1972). The fact that there is no diurnal variation in oestradiol concentration while testosterone shows distinct peaks in the early morning may imply that the rise in oestradiol concentrations in puberty may be due to an independent increase in testicular secretion rather than peripheral conversion of testosterone. Although the above data lend themselves to various interpretations, it is resonable to speculate that in male as in female puberty the rise in testicular oestradiol secretion is responsible for the small prolactin rise observed in this study, even though the temporal correlation between the two is rather poor. If that is the case it follows that prolactin is unlikely to have an active trophic effect on gonadal development in the human.

This study attempted to investigate the gonadal mechanisms in puberty in man by relating the diurnal patterns in circulating reproductive hormones to the known morphological and functional changes in the developing testis. Although direct extrapolation of experimental findings in lower animals to the human is inadvisable, it is nevertheless reasonable to interpret some of the present data in the light of the current concepts of Leydig cell regulation. Although significant advances have been made in the last five years in our understanding of the regulation of Leydig cell functions, a great deal remains to be learned about the control of Leydig cells in the initiation of spermatogenesis during pubertal maturation.

In summary, this study confirmed the role of LH and FSH in the development of adult Leydig cell function during puberty. Testicular steroidogenesis during puberty operates with little functional reserve. This may be one of the explanations of the lack of a distinct pulsatile pattern in circulating testosterone. The role of prolactin in puberty and the physiological significance of a transient rise in plasma oestradiol remain unclear.

CHAPTER 6

DIAGNOSIS AND MANAGEMENT OF DELAYED PUBERTY

6.1 Introduction

Hypogonadal disorders first manifest themselves at the time of adolescence when signs of puberty fail to appear. Although the simultaneous presence of multiple trophic hormone deficiencies or somatic defects such as anosmia can facilitate the diagnosis of gonadotrophin deficiency, in the majority of cases it is impossible to differentiate between isolated hypogonadotrophic hypogonadism and simple constitutional delayed puberty during the early years of adolescence on clinical grounds alone. Both groups of patients present with lack of sexual development associated with low gonadotrophin and sex steroid production so that basal measurements of these hormones are unhelpful. Some reports have described minimal elevations in basal plasma gonadotrophin concentrations before the clinical onset of puberty (Johanson et al, 1969; Winter & Faiman, 1972). However, the small magnitude of this initial rise and the low values of gonadotrophins near the assay detection limit render their interpretation hazardous.

The availability of synthetic GnRH and its successful application to the study of pituitary responsiveness in children has provided an additional diagnostic test in the management of disorders of puberty. Early reports confirmed the increasing gonadotrophin reserve of the

pituitary during normal pubertal development (Kastin et al, 1972; Job et al, 1972; Roth et al, 1972) raising the possibility that the GnRH stimulation test may resolve the diagnostic difficulties in subjects with delayed puberty. Further studies however revealed that the pituitary response to GnRH in normal children and adolescents varied considerably within a wide range showing substantial overlap between prepubertal and pubertal subjects. Furthermore, the GnRH responses amongst patients with organic hypogonadotropic disorders were also variable and unpredictable, making the differentiation between primary hypothalamic and pituitary abnormalities impossible (Bell et al, 1973; Madeiros-Neto et al, 1973; Mortimer et al, 1973; Chaussain et al, 1974; Coscia et al, 1974; Grumbach et al, 1974; for review see Job et al, 1977). Despite these drawbacks, several studies claimed that the GnRH response may distinguish between constitutional delayed puberty and isolated gonadotrophin deficiency (Illig et al, 1973; Grumbach et al, 1974; Sagel et al, 1975; Franchimont et al, 1975), stressing the prognostic value of this test. This was not confirmed by Job et al (1976 & 1977) who found no clear-cut separation between these two conditions in terms of their pituitary responses to single-dose GnRH stimulation. The application of more prolonged pituitary stimulation by continuous GnRH infusion (Bremner & Paulsen, 1974; Kley et al, 1974; de Kretser et al, 1975a) or multiple GnRH bolus stimulation (Wang et al, 1975; Hoff et al, 1977) consistently obtained not only a

greater FSH response but also a biphasic LH response which may represent two functional components of pituitary gonadotrophin production (see Chapter 4). Continuous GnRH infusion in children showed that the biphasic pattern of LH response could only be detected in pubertal but not prepubertal subjects (de Lange et al, 1974; Reiter et al, 1976; Huseman & Kelch, 1978). Prolonged or repetitive GnRH stimulation tests may thus improve on the diagnostic accuracy of the single dose version in the assessment of the functional maturity of the hypothalamus/pituitary in disorders of puberty.

The value of GnRH tests in the management of delayed puberty can be assessed in two ways. The most obvious is to compare the GnRH response in patient groups whose clinical outcome and eventual diagnoses are known. This is the approach used in all previous studies (Illig et al, 1973; Grumbach et al, 1974; Franchimont et al, 1975; Job et al, 1976). The alternative is to correlate the pituitary response to exogenous GnRH in these subjects to other changes in hypothalamic-pituitary function which characterize the onset and progression of pubertal development. The nocturnal sleep-related gonadotrophin secretion first described by Boyar et al (1972b) is now generally accepted as a precise biological marker of pubertal onset in humans (Boyar et al, 1973a & b; Weitzman et al, 1975; Parker et al, 1975; Judd et al, 1977; Giusti et al, 1979). This early indicator of puberty is detectable in late prepubertal children prior to the onset of clinical puberty (Lee et al, 1976; Judd

et al, 1977; Boyar, 1978; Giusti et al, 1979) and the nocturnal rise in gonadotrophin is believed to be responsible for stimulating the gonads to synthesize and secrete sex steroids in a diurnal pattern during sexual maturation (Boyar et al, 1974a; Judd et al, 1974; Parker et al, 1975). If the pituitary response to exogenous GnRH during pubertal development can be related to nocturnal gonadotrophin secretion as well as the eventual clinical diagnosis, the diagnostic and prognostic usefulness of both these hormonal parameters in the management of delayed puberty may be better understood.

These considerations prompted us to study the nocturnal gonadotrophin secretion and the GnRH response simultaneously in a group of boys with impaired or delayed puberty whose progress was followed for up to 39 months. Pituitary responsiveness was assessed by multiple submaximal doses (10 μg) of GnRH administered as I.V. boluses at two hourly intervals in order to mimic the nocturnal endogenous GnRH pulsatile stimulation of the pituitary.

6.2 Results

The individual values of mean plasma LH and FSH concentrations, spontaneous LH pulse frequency and amplitude and the maximal incremental and integrated area response to the four 10 μg bolus of GnRH are shown in Table 4.1. Kendall's rank correlation coefficients between each of the thirteen LH and nine FSH measures relevant to this section are illustrated in Table 6.1

			Night LH pulses		Evening LH pulses		Mean LH			Δ Max LH u/l		GnRH Response	Integrated Area LH Area Units	
			Frequency	Amplitude	Frequency	Amplitude	Evening	Night	Morning	First pulse	Mean of 2nd to 4th pulse	Mean of 4 pulses	First pulse	Mean of 2nd to 4th pulse	Mean of 4 pulses
Night LH Pulses	Frequency			0.28	0.34	0.20	0.29	0.40	0.41	0.42	0.40	0.45	0.45	0.40	0.40
	Amplitude		0.28		0.53	0.62	0.60	0.69	0.54	0.67	0.53	0.54	0.64	0.54	0.60
Evening LH Pulses	Frequency		0.34	0.53		0.77	0.53	0.48	0.42	0.42	0.45	0.47	0.38	0.42	0.42
	Amplitude		0.20	0.62	0.77		0.60	0.42	0.31	0.43	0.33	0.34	0.39	0.33	0.36
Mean LH u/l	Evening		0.29	0.60	0.53	0.60		0.59	0.52	0.51	0.43	0.44	0.46	0.46	0.51
	Night		0.40	0.69	0.48	0.42	0.59		0.56	0.64	0.60	0.64	0.61	0.60	0.64
	Morning		0.41	0.54	0.42	0.31	0.52	0.56		0.56	0.59	0.58	0.56	0.68	0.66
	First pulse		0.42	0.67	0.42	0.43	0.51	0.64	0.56		0.58	0.65	0.86	0.59	0.69
Δ Max LH u/l	Mean of 2nd to 4th pulse		0.40	0.53	0.45	0.33	0.43	0.60	0.59	0.58		0.94	0.58	0.83	0.84
	Mean of 4 pulses		0.45	0.54	0.47	0.34	0.44	0.64	0.58	0.65	0.94		0.63	0.82	0.85
GnRH Response	First pulse		0.45	0.64	0.38	0.39	0.46	0.61	0.56	0.86	0.58	0.63		0.61	0.73
Integrated Area LH Area Units	Mean of 2nd to 4th pulse		0.40	0.54	0.42	0.33	0.46	0.60	0.68	0.59	0.83	0.82	0.61		0.89
	Mean of 4 pulses		0.40	0.60	0.42	0.36	0.51	0.64	0.66	0.69	0.84	0.85	0.73	0.89	

Table 6.1

Kendall rank correlation coefficients between nocturnal and evening LH pulse frequency (per 10 hours) and amplitude (u/l), mean evening, nocturnal and morning plasma LH concentrations (u/l) and the LH maximal incremental (Δ Max) and integrated area response to the four consecutive 10 μg bolus of GnRH at 2 hourly intervals in 16 prepubertal and pubertal subjects studied on 30 occasions.

		Mean FSH u/l			Δ Max FSH u/l		GnRH Response	Integrated Area FSH Area Units	
		Evening	Night	Morning	1st pulse	Mean of 2nd to 4th pulse	Mean of 4 pulses	1st pulse	Mean of 2nd to 4th pulse	Mean of 4 pulses
Mean FSH u/l	Evening		0.78	0.78	0.28	0.16	0.23	0.32	0.15	0.25
	Night	0.78		0.87	0.27	0.20	0.27	0.27	0.21	0.27
	Morning	0.78	0.87		0.23	0.21	0.25	0.25	0.21	0.25
Δ Max FSH u/l	First pulse	0.28	0.27	0.23		0.26	0.44	0.83	0.27	0.43
	Mean of 2nd to 4th pulse	0.16	0.20	0.21	0.26		0.85	0.21	0.82	0.74
	Mean of 4 pulses	0.23	0.27	0.25	0.44	0.85		0.36	0.82	0.85
GnRH Response	First pulse	0.32	0.27	0.25	0.83	0.21	0.36		0.24	0.43
Integrated Area FSH Area Units	Mean of 2nd to 4th pulse	0.15	0.21	0.21	0.27	0.82	0.82	0.24		0.82
	Mean of 4 pulses	0.25	0.27	0.25	0.43	0.74	0.85	0.43	0.82	

Table 6.2

Kendall rank correlation coefficients between mean evening, nocturnal and morning plasma FSH concentrations (u/l) and the FSH maximal incremental (Δ Max) and integrated area response to the four consecutive 10 μg bolus of GnRH at 2 hourly intervals in 16 prepubertal and pubertal subjects studied on 30 occasions.

and 6.2. The mean nocturnal plasma LH concentration and nocturnal pulse amplitude were best correlated with the pituitary response to the first GnRH bolus or pulse (Δ Max, r = 0.64 + 0.67 and Σ area, r = 0.61 + 0.64 respectively (Kendall's rank correlation). The mean of the second to the fourth or of all four consecutive LH responses to exogenous GnRH pulses correlated with the two measures of nocturnal LH secretion less well (r = 0.53-0.64). Nocturnal LH pulse frequency was poorly correlated with either mean basal gonadotrophin levels or the GnRH response. Nocturnal plasma FSH concentration was best correlated with the morning levels (r = 0.87).

The maximal LH incremental response and integrated area under the response curve to GnRH stimulation were significantly correlated (r = 0.86 for first pulse; r = 0.82 for the mean of second to fourth pulses and r = 0.85 for the mean of four pulses). Similarly the pituitary LH response to the first GnRH bolus correlated with the mean response to four consecutive 10 μg bolus (r = 0.65 for Δ max and r = 0.73 for Σ area) but less so with the mean response to the second to fourth boluses. For FSH, although the maximal incremental and integrated responses were highly correlated (first pulse r = 0.83, second to fourth pulse r = 0.79, all four pulses r = 0.85) the response to the first bolus was only poorly correlated with that to the subsequent stimuli (r = 0.24-0.44).

The linear relationships between mean nocturnal LH concentration and nocturnal LH pulse amplitude on the one

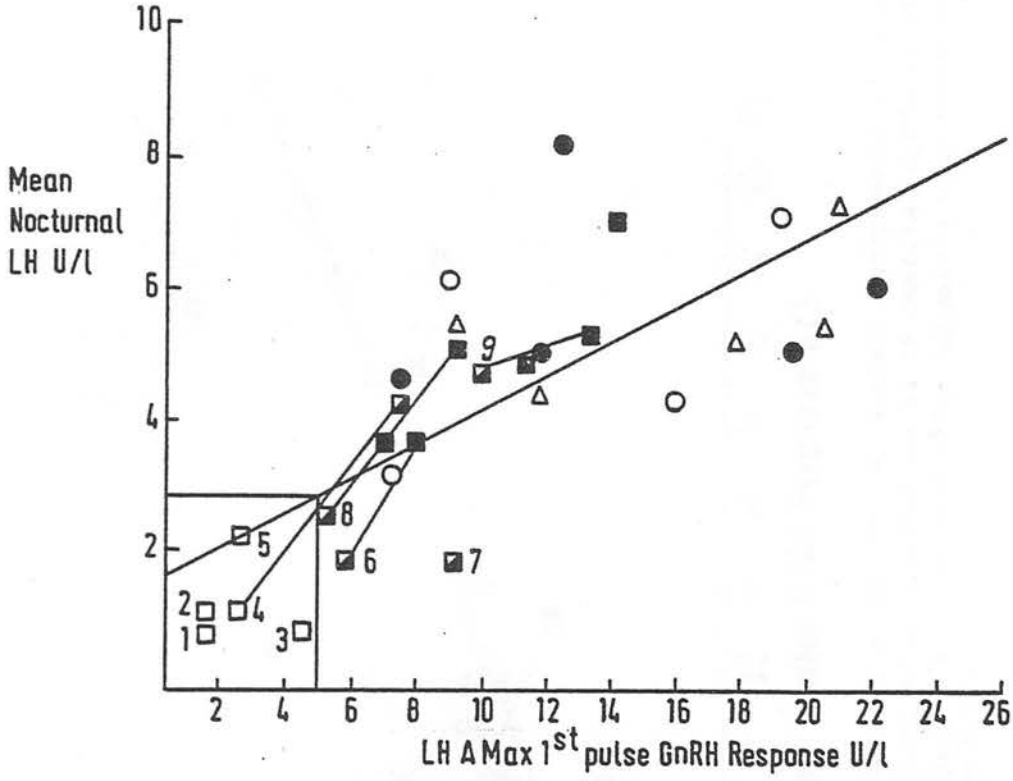


Fig 6.1

The relationship between mean nocturnal LH concentration and the maximal incremental (Δ Max) LH response to the first of the four 10 μg GnRH bolus stimulation in 16 pubertal subjects studied on 30 occasions. Linear regression analysis: $y = 1.45 + 0.263x$; Kendall's rank correlation $\tau = 0.64$, $p<0.001$. Puberty stage 1A = □, stage 1B = ◪, stage 2 = ■, stage 3 = ○, stage 4 = ● and stage 5 = Δ. Solid lines indicate follow-up studies in the same pre- or peripubertal subject approximately 6 months after the initial one. All the subjects with a GnRH-induced LH increment of 5 u/l or more were either established in puberty at the time of study or progressed into puberty on follow-up. Although subject 7 did not undergo a second sleep study, his subsequent pubertal development was satisfactory. Neither the diagnosis nor the prognosis could be determined in those subjects with a low (LH increment <5 u/l) GnRH response.

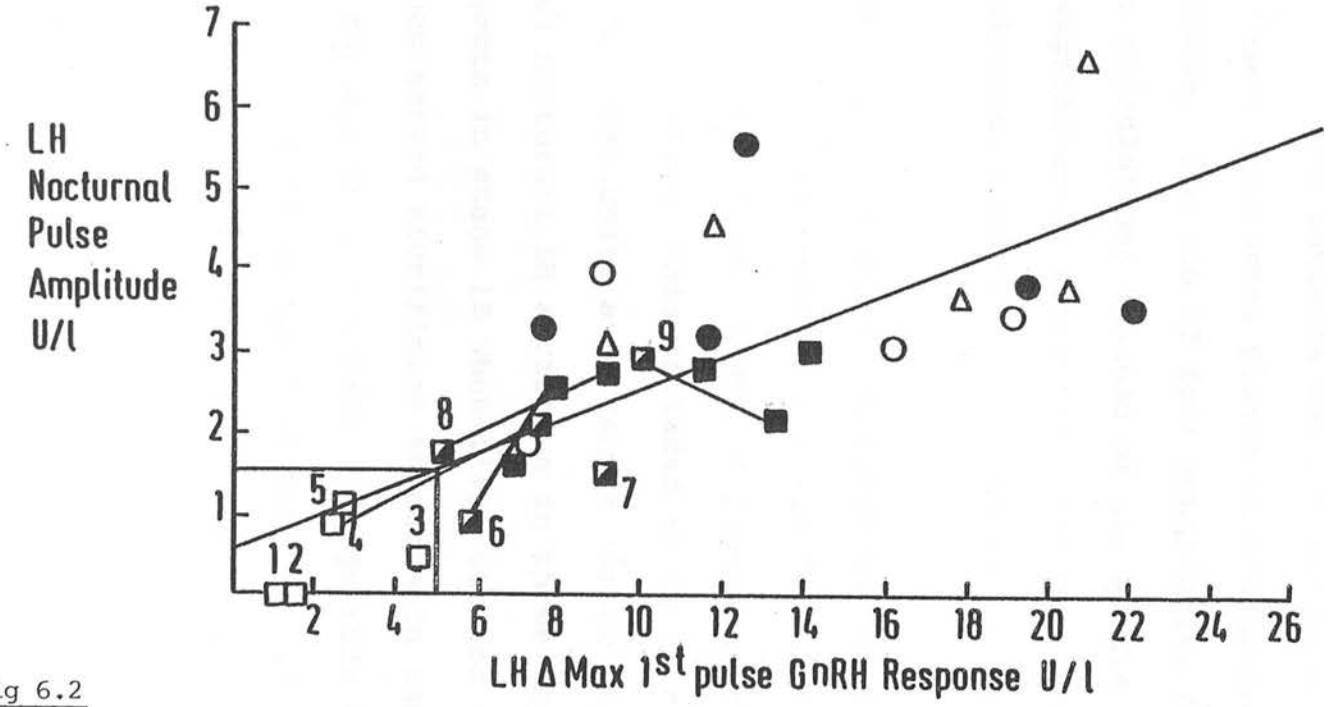


Fig 6.2

The relationship between nocturnal LH pulse amplitude and the maximal incremental (Δ Max) LH response to the first of the four 10 μg GnRH bolus stimulation in 16 pubertal subjects studied on 30 occasions. Linear regression analysis - $y = 0.60 + 0.194x$; Kendall's rank correlation $\tau = 0.67$, $p<0.001$ (also see legend to Fig 6.1).

hand and the maximal LH incremental response to the first GnRH bolus on the other are shown in Figures 6.1 and 6.2. Stage 1A prepubertal patients generally had lower values for both nocturnal gonadotrophin measures and smaller GnRH response. The considerable overlap of individual LH values in different clinical stages of puberty especially in the more mature patients was not minimized by combining basal nocturnal plasma LH measurements with the GnRH response. The use of four consecutive GnRH pulsatile stimulations instead of the single dose test did not significantly alter the distribution of the various clinical pubertal groupings (data not shown in figures).

Maximal LH incremental response to the first 10 μg GnRH stimulation exceeding 5 u/l was found in all patients who showed some signs of pubertal development when first studied. This included those four subjects with minimal testicular enlargement (Stage 1B). Despite the lowish nocturnal LH secretion in three out of the four subjects in stage 1B when first studied (Fig 6.1), all of them showed significant advances in the clinical pubertal ratings or an increase in magnitude of nocturnal LH secretion or both in the following six to twelve months (Figs 6.1 & 6.2, also see Table 2.1). One of these patients (subject 8), a growth-hormone deficient individual with low I.Q., showed a dramatic maturation in his gonadotrophin secretory pattern (see profiles in Appendix II) between the first and second study with a six-month interval. Nevertheless, androgen treatment was

instituted shortly after the second study due to increasing parental and social pressures.

All patients in stage 1A had a maximal LH incremental response to the first GnRH bolus less than 5 u/l. One of these (subject 4) with growth hormone deficiency showed a substantial increase in nocturnal LH secretion and pituitary responsiveness when re-studied six months later (Figs 6.1 & 6.2). Again androgen treatment had to be commenced shortly afterwards because of his advancing age and the fact that he was leaving school to seek employment. A further patient in stage 1A (subject 5) received a twelve week course of hCG (Pregnyl) 3000 I.U. twice weekly immediately following the first study. Three months after the termination of the treatment course (six months from the first study) the gonadotrophin and testosterone profile (Fig 4.1) showed a dramatic maturation and this patient subsequently developed to full sexual maturity with no further treatment. Although this patient (subject 5) underwent serial hormonal studies on four occasions in a 26-month period of follow-up (Appendix II) the subsequent three studies had not been included in the group data analyses because of the possible confounding effects of the twelve week course of treatment. The other three patients in stage 1A (subjects 1, 2 and 3) were characterized by insignificant nocturnal LH secretion, low GnRH response even on repeated stimulation and somatic abnormalities such as anosmia, colour blindness, cryptorchidism and deafness (Table 2.1). In the two

younger patients, one of whom is also growth hormone deficient (subject 2), no significant sexual development occurred spontaneously in the subsequent twelve months following which testosterone replacement was instituted. The older patient with Kallmann's syndrome was started, immediatedly after his sleep study, on Sustanon injections and later changed to hCG with HMG after he married. On the evidence from these five patients, the LH response to GnRH stimulation either by single or multiple doses was unable to differentiate the three definitely hypogonadotrophic patients from the other two which showed significant advancement in hypothalamic-pituitary function six months later. Interestingly, the nocturnal plasma LH concentration and pulse amplitude were both slightly higher in the latter two patients.

The rest of the study group consisted of patients with constitutional delayed puberty in more mature stages of development. All of these patients advanced their pubertal ratings in the 39-month period of follow-up even though not all of them had repeat hormonal studies. Five patients (subjects 12, 13, 14, 15 and 16) with constitutional delayed puberty reached full maturity at the end of the study (Table 2.1).

6.3 Discussion

Pubertal maturation involves the programmed and sequential development of the brain, hypothalamus, pituitary and gonads - the chemical messengers produced at each level in maturation being responsible for

activating the next (Styne & Grumbach, 1978; Grumbach, 1980). Investigations of puberty initiation should therefore concentrate on the most proximal or highest parts of this functional hierarchy. Unfortunately, our efforts at present are limited to studying the part of the brain-hypothalamus-pituitary-gonadal axis distal to hypothalamic GnRH which is present in barely detectable amounts in the peripheral circulation (Arimura et al, 1974; Jeffcoate et al, 1975; Kelch et al, 1975; Mortimer et al, 1976). It has therefore been necessary to resort to indirect tests of endogenous hypothalamic GnRH secretion in the investigations of pubertal disorders. Ideally these investigations should aim to evaluate the degree of hypothalamic function from the time when it is activated and also to predict that progression to full maturity will take place.

The pituitary response to exogenous GnRH is said to reflect the degree of previous priming of the gonadotropes by endogenous hypothalamic GnRH (Grumbach et al, 1974; Mortimer et al, 1975; Hashimoto et al, 1975; Reiter et al, 1975 & 1976; Boyar et al, 1976). Since hypothalamic GnRH secretion is believed to be pulsatile (Sarkar et al, 1976; Carmel et al, 1976; Neil et al, 1977; Eskay et al, 1977) a multiple submaximal dose GnRH stimulation protocol was adopted in an attempt to reproduce the endogenous pattern and to study the dynamics of pituitary gonadotrophin secretion in terms of an immediately releasable first pool and a newly synthesized reserve second pool (Yen et al, 1975; Yen,

1977). In conjunction, the episodic gonadotrophin secretion during sleep in late prepubertal and pubertal children was used to provide a fairly precise index of hypothalamic GnRH secretion during puberty (Boyar et al, 1976; Boyar, 1978).

The results in this study confirmed that both nocturnal LH secretions and the pituitary response elicited by multiple low-dose pulsatile stimulation progressively increased in magnitude from prepuberty to adulthood. Consequently, both parameters of gonadotrophin production can apparently be regarded as valid indices of pubertal maturation of hypothalamic function. However, nocturnal LH levels and pulse amplitude as well as the GnRH response were all rather variable. Individual hormone values showed considerable overlap in consecutive and even non-consecutive stages of puberty especially amongst the more mature subjects. It follows that neither nocturnal LH nor the GnRH response were adequate in identifying individual pubertal subjects at specific stages of maturity. Nevertheless, the latter investigation may, within certain confines, be successfully deployed to follow serially the progress of individual patients over a period of time (see later). The variability of pituitary gonadotrophic function in this study is in accordance with that found in previous reports (Job et al, 1977; Sizonenko, 1978 for review) and is perhaps to be expected in view of the inherent variability in onset and duration of specific stages of puberty in different individuals (Marshall & Tanner, 1970).

The pituitary LH response to four consecutive boluses of GnRH assessed by incremental maximal or integrated area under the dose-response curve provided the same information. The response to the first GnRH bolus correlated closely with the responses to the subsequent three boluses, so that they displayed similar relationships to the parameters of nocturnal LH secretion. Thus the repetitive mode of pituitary stimulation did not contribute any additional information clinically to that obtainable from the single dose (10 μg) stimulus. It can therefore be concluded that the best and most convenient hormonal parameter for the clinical assessment of pituitary responsiveness and indirectly hypothalamic function in puberty is the maximal incremental LH response to the first 10 μg bolus of GnRH.

The fixed dose of 10 μg selected for repeated sub-maximal challenge of the pituitary (Rebar et al, 1973) delivered between 170-340 ng/Kg body weight of GnRH to individual patients in this group. Within this range, the trend was for the more mature boys to receive slightly lower amounts of GnRH per unit body weight. This has not prevented the demonstration of the progressive rise in pituitary responsiveness during puberty similar to that described in previous studies (reviewed in Job et al, 1977). Judging from the dose-response studies of Grumbach et al (1974), the small differences in GnRH dosage per unit weight in this study is unlikely to have made any significant differences to the results obtained.

A maximal LH incremental response to the first 10 μg bolus of GnRH of 5 u/l or more in this study is usually compatible with the presence or imminent onset of significant nocturnal gonadotrophin secretion and testicular volumes of at least 3 mls or more. The vast majority of patients in this category will progress to full sexual maturity spontaneously. These patients are considered to have constitutional delayed puberty. In those with a GnRH response of under 5 u/l of LH, it is not possible to differentiate between those who will develop spontaneously from others with hypogonadotrophic hypogonadism. The eventual diagnosis will only become apparent by performing follow-up hormone studies or monitoring the progress of the individual patient over a period of time. In some cases, an unequivocal diagnosis may not be possible. In two patients (subjects 4 and 8) the clinical situation demanded that rapid virilization can be induced by treatment even though there was definite evidence of spontaneous development. It is interesting that both these patients were deficient in growth hormone as well. They had been receiving growth hormone replacement for at least two years prior to the present study, but this is unlikely to influence either the pituitary response to GnRH or the eventual outcome of pubertal development (Kelch et al, 1976). That these two patients, despite the presence of significant nocturnal LH secretion and adequate GnRH response on follow-up study, were still barely into puberty is contrary to the findings of Sauder et al (1981). These

workers found subnormal LH concentrations and GnRH response in boys with isolated growth hormone deficiency even after normal progression of sexual maturation. It may be possible that growth hormone deficient boys may secrete LH with both greater (Lucky et al, 1980) or lesser (as in our patients) than normal biological to immunological potency ratio. Whatever may be the case, this further emphasizes the limitation of both the GnRH test and nocturnal LH concentrations in the diagnosis of hypogonadotrophism in boys with growth hormone deficiency. Nevertheless, in the third patient in this series with growth hormone deficiency (subject 2), there was little doubt regarding the associated gonadotrophin deficiency. Apart from the immature pattern of gonadotrophins, he had a past history of encephalitis, multiple congenital somatic defects and a complete lack of clinical progress in the following twelve months.

Pubertal development should be regarded as a reactivation of the hypothalamic-pituitary-testicular axis (Grumbach, 1980). In males with normally differentiated external and internal genitalia, normal prenatal function of the hypothalamic-pituitary-testicular axis is implied (Jost, 1972; Ohno, 1976; Wilson, 1978). In patients with delayed pubertal development, most of whom have normally differentiated genitalia, the hypothalamus, pituitary and testes must have had the potential for normal function given the suitable stimulation at the appropriate time. Delayed secondary sexual maturation in most instances must reside in the

defective or delayed CNS reactivation of the hypothalamic signal generator. It is conceivable that the complex bimodal nature of sexual development in humans increases the likelihood of functional abnormalities especially in the reactivation phase giving rise to clinical disorders such as delayed puberty or hypogonadotrophic hypogonadism. It may be relevant that girls, whose onset of puberty is approximately two years earlier than boys, have a much lower incidence of delayed puberty. One interpretation of these observations is that simple delayed puberty and hypogonadotrophic hypogonadism are extreme examples of a spectrum of CNS-hypothalamic abnormalities where the stimulus for hypothalamic GnRH synthesis and release is either temporarily delayed or permanently deranged. This concept is supported by the heterogeneous pattern of gonadotrophin secretion in patients with idiopathic gonadotrophin deficiency (Bell et al, 1973; Zarate et al, 1973; Boyar et al, 1973 & 1976; Spitz et al, 1974) resulting from different degrees of endogenous GnRH deficiency. This may have arisen from the failure of initiating or of the continuing progression of neuro-secretory mechanisms which activate the hypothalamus. Constitutional delayed puberty, as its name implies, is simply due to the later attainment of CNS maturation necessary for the initiation of the peripubertal hypothalamic GnRH secretion. This is compatible with the clinical observation that earlier growth patterns in boys with constitutional delayed puberty are frequently at or below the third percentile of the normal range

(Prader, 1975; Brooks, 1981) so that their overall growth and physical development are relatively retarded. It is also relevant that patients with isolated growth hormone deficiency frequently have chronologically delayed sexual maturation but they enter puberty at a normal bone age (Tanner & Whitehouse, 1975).

Accepting that constitutional delayed puberty and idiopathic hypogonadotrophic hypogonadism share common pathophysiological mechanisms varying only in timing and severity, it should therefore not be surprising that both the pituitary responsiveness and the nocturnal GnRH secretion are so variable and unpredictable in patients with delayed sexual maturation. Both these parameters are seldom characteristic of specific physiological or pathological states but they simply reflect the extent of hypothalamic development and the degree of previous exposure of the pituitary to endogenous GnRH. Neither the pituitary response to GnRH nor the measurement of nocturnal gonadotrophin secretion in prepubertal subjects without serial determinations over a reasonable duration of observation can be expected to have much predictive or prognostic value in individual patients. In practice, some patients with extreme delay in pubertal onset or unusually slow progression from the early stages of puberty are often treated by sex hormones regardless of the definitive diagnosis. This further emphasizes that the distinction between constitutional delayed puberty and hypogonadotrophic hypogonadism is not always possible and may sometimes depend on arbitrary clinical

definitions or yield to overriding therapeutic considerations.

In summary, this study showed that the pituitary response to exogenous GnRH can differentiate between subjects in late prepuberty or early puberty from those in early prepuberty. In the latter group, however, the GnRH response cannot differentiate between patients with gonadotrophin deficiency from those with constitutional delayed puberty. The assessment of nocturnal gonadotrophin secretion and the use of multiple-dose GnRH stimulation did not confer any additional diagnostic or prognostic accuracy. The limitation of gonadotrophin measurement, whether basal or stimulated, in patients with growth hormone deficiency was discussed.

SECTION C

STUDIES IN MALE INFERTILITY

CHAPTER 1

INTRODUCTION

In the previous section, studies on the development and control of testicular functions were based on the measurement of testosterone and testicular volume. Although Leydig cell steroidogenesis can be adequately assessed by circulating testicular steroid concentrations, spermatogenesis in adolescent subjects is less amenable to investigations. As mentioned, testicular volume is a valid but rather crude index of spermatogenesis. The detection of spermaturia (Richardson & Short, 1978), though convenient, is subject to a significant incidence of false negative and is probably masturbation-related. Obviously, semen analysis and testicular biopsy in these pubertal patients are not practical nor can they be ethically justified. It follows that seminiferous tubule functions may be more fruitfully investigated in adult subjects.

The study of hormonal control of spermatogenesis is complicated by the presence of different functional/anatomical components within the seminiferous tubules (see Section A, Chapter 8) and the lack of convenient parameter(s) of Sertoli cell functions. In addition, the ill-defined functional interrelationship between Sertoli cells and germ cells further complicates the picture. Although not all these difficulties can be bypassed, one approach to the study of the endocrine control of spermatogenesis is to examine the hormonal changes

resulting from primary seminiferous tubule damage in infertile men. This provides an opportunity not only to assess the value of further diagnostic parameters in conjunction with semen analysis, but also to explore the functional interrelationships between spermatogenesis, gonadotrophins and testicular steroidogenesis. The latter includes, apart from androgens, the synthesis and secretion of oestrogens (Kelch et al, 1972) for which no definite physiological function has been ascribed. FSH can stimulate the production of oestradiol in cultured rat Sertoli cells (Dorrington & Armstrong, 1975) while oestrogens preferentially suppress FSH secretion in vivo (Kulin & Reiter, 1972). These findings seem to imply that oestrogens, under certain circumstances, may have a similar spectrum of biological properties to inhibin - the putative seminiferous tubular factor which selectively suppresses FSH. A further aspect of testicular oestrogens is the possibility that they may play a role in the intratesticular control of Leydig cell function (Dufau et al, 1978). This is of particular interest in view of the evidence that androgen production may be impaired in infertile men (de Kretser et al, 1972). The altered endocrine environment created by seminiferous tubular damage in infertile men afforded a unique opportunity to evaluate the possible physiological functions or pathophysiological significance of testicular oestrogens.

It has been estimated that 10-15% of marriages experience difficulty in producing children (Behrman & Kistner, 1975). In 20% of childless marriages, the

abnormality lies solely with the husband and in a further 20% both husband and wife contribute to the infertility (Buxton & Southam, 1958). The male causes of infertility are mainly due to defects in the semen such as oligospermia, azoospermia, poor motility and abnormal morphology (Hotchkiss et al, 1938). The American Fertility Society recommended that the lower limit of normal sperm density is 40 million/ml, but it is now widely accepted that there is little increase in fertility with sperm densities above 20 million/ml if the other semen parameters are constant (Macleod & Gold, 1951). Other authors were able to demonstrate a statistically significant reduction in pregnancy rate only when the sperm density has fallen below 10 million/ml (Smith et al, 1977; Sherins et al, 1977). It is obvious that the assessment of fertility potential by semen analysis is based on the principle of probability and the diagnosis of male subfertility cannot be absolute. Thus any male who is not azoospermic will stand some chance of fecundity and subjects with normal semen parameters are not necessarily fertile (e.g. in the presence of high titres of anti-sperm antibodies). Barriers to fertilization may also result from sexual difficulties, penile abnormalities or other extraneous factors such as infections and use of certain lubricants. The aetiological factors in male infertility are listed in Table 1.1. Despite the numerous factors that may disrupt the functions of the seminiferous tubules and accessory glands, the aetiological factor(s) in over 50% of patients with

Table 1.1 Aetiological factors in male infertility

Defects in Technique

Infrequent and poor timing of intercourse

Impotence and ejaculatory failure

Use of sperm-toxic lubricants

Testicular Factors

Idiopathic

Chronic illness - renal failure, hepatic failure, malnutrition

Cryptorchidism

Varicocoele

Orchitis - Mumps

Trauma, torsion

Heat - occupation or local

Toxic chemicals and cytotoxic drugs

Chromosomal disorders - Klinefelter's (47XXY) syndrome

Irradiation

Endocrine deficiency - hypogonadotrophic hypogonadism

Androgen end-organ insensitivity

Outflow Tract/Accessory gland pathology

Agenesis of vasa deferentia and seminal vesicles

Obstruction of epididymis or vas

Prostatovesiculitis

Anti-sperm antibodies

infertility are unknown. However, with the rare exceptions of those with hypogonadotrophic hypogonadism and chronic illnesses, testicular factors are responsible for the vast majority of cases of male infertility (Van Zyl et al, 1975; de Kretser, 1979). Irrespective of the nature of the individual testicular aetiological factors in the infertile male, the end result is the depletion of germ cells and/or arrest of their maturation, producing failure of spermatogenesis. The hormonal changes resulting from injury to the seminiferous epithelium are the subject of the following studies.

CHAPTER 2

ENDOCRINE ASSESSMENT OF THE SUBFERTILE MALE

2.1 Introduction

In assessing the reproductive capacity of the human male, it is not feasible, as it is in animal husbandry, to test the fertility potential of an ejaculate against multiple female recipients. In vitro tests of fertilization, though a promising research technique, has not reached the stage of routine clinical use as yet. Semen analysis has therefore been the mainstay in fertility assessment in men (Eliasson, 1975) even though there is no direct evidence that the commonly measured semen characteristics are critically related to the fertilizing potential of ejaculates in humans. Furthermore, because of the inherent variability in testicular sperm production (Freund, 1963) and the difficulties in standardizing sample collection (Freund, 1962), the accuracy and precision of semen analysis fall short of the ideal. Consequently, there is no clear cut demarcation between a normal and an abnormal ejaculate and the prediction of fertility or infertility based on semen analyses can at best only be approximate (Eliasson, 1975; Rehan et al, 1975; Sherins et al, 1977). Other methods of investigations have therefore been employed to complement semen analysis in the assessment of male subfertility. Testicular biopsy has been a useful way of directly assessing the morphological state of the seminiferous epithelium (Charny, 1940; Hotchkiss, 1942; Nelson,

1953; Rosen-Runge, 1956; Johnsen, 1970a; Meinhard et al, 1973; Wong et al, 1973; Aafjes et al, 1974 & 1978). However, considerable expertise and time are required in the meaningful quantitative interpretation of testicular histology and the procedure is not without morbidity. With the wider availability of hormone assays, the measurement of FSH and other reproductive hormones has added a new dimension to the investigation of male subfertility. Several studies reported a reciprocal relationship between urinary or plasma FSH and the number of germ cells in seminiferous tubule cross sections (Johnsen, 1970b; Franchimont et al, 1972; de Kretser et al, 1974; Baker et al, 1976). Sperm density has also been shown to have an inverse relationship to FSH (Rosen & Weintraub, 1971; Mauss & Börsch, 1973; Kjessler & Wide, 1973; Hunter et al, 1974; Christiansen, 1975; Aafjes et al, 1977). This however was not confirmed by others (Leonard et al, 1972; Franchimont et al, 1972; de Kretser et al, 1972). Thus the role of plasma FSH measurement as a quantitative index of seminiferous tubule function and its relationship to histological changes in testicular biopsies in male infertility are not entirely clear. We therefore decided to further evaluate the efficacy of plasma FSH in assessing the degree of seminiferous tubule damage at different levels of sperm density in men presenting with infertility.

GnRH has been used in dynamic tests of the hypothalamic-pituitary-testicular axis in a variety of hypogonadal conditions (Franchimont et al, 1975).

Exaggerated FSH response to GnRH proportional to basal levels has been reported in patients with oligospermia and azoospermia (Isurgi et al, 1973; Mecklenberg & Sherins, 1974; Franchimont et al, 1975; Roulier et al, 1976; Guay et al, 1977). However, Lipschultz et al (1977) found exaggerated FSH response to GnRH in oligospermic patients with normal basal FSH levels. This implied that the GnRH response (rather than basal FSH) may be a more sensitive index of seminiferous tubular function. The clinical value of the GnRH test in comparison with basal FSH measurements in male subfertility therefore needs further evaluation.

Although clinically apparent degrees of androgen deficiency are uncommon in subfertile males, evidence of Leydig cell dysfunction can be detected in those with more severe forms of seminiferous tubular damage. This is suggested by elevated basal LH (Kjessler & Wide, 1973; Mauss & Börsch, 1973; Christiansen, 1975; Hunter et al, 1974), exaggerated LH response to GnRH stimulation (Isurgi et al, 1973; Mecklenberg & Sherins, 1974; de Kretser et al, 1975; Roulier et al, 1976; Guay et al, 1977) and more directly indicated by statistically lower testosterone levels or impaired response to hCG stimulation (de Kretser, 1972 & 1975b). Study of the LH-testosterone axis may therefore also furnish diagnostically useful information in addition to FSH measurements in the investigation of male fertility status. Since LH and testosterone, and to a lesser extent FSH, are secreted episodically (Nankin & Troen, 1971; Naftolin et

al, 1973; Santen & Bardin, 1973; Alford et al, 1973; Smith et al, 1974) multiple measurements of these hormones may provide more accurate information than single estimations.

The aims of the present study are to evaluate and compare the clinical application of (1) single basal measurements of FSH, LH and testosterone; (2) multiple measurements of FSH, LH and testosterone and (3) the GnRH response, in the assessment of subfertile males in terms of their relationship with testicular histology and sperm production. Having established the best hormone parameter(s) and their limitations, the approach to investigation of the subfertile male and the indications for testicular biopsy can then be rationalized.

2.2. Patients and methods

2.2.1 Patients

A total of 103 consecutive patients referred to the Male Subfertility Clinic, Royal Infirmary, Edinburgh with average sperm density under 40 million/ml were studied. All patients were normally virilized and had normal male chromosome karyotype (46XY). The median age was 34 and range 24-45 years. The clinical histories, summarized in Table 2.1, revealed no abnormalities in three quarters of the group. A history of unilateral or bilateral cryptorchidism could be elicited in eleven patients while six had infections in the genital tract such as mumps, gonorrhea and nonspecific urethritis. One patient received radiotherapy to the spine for ankylosing spondy-

History		Physical Findings	
Nil of note	82	Normal external genitalia	51
Unilateral undescended or retractile testis	6	Bilateral small testes	27
Bilateral undescended or retractile testes	5	Unilateral small testis	7
Genital infections	6	Varicocoele	12
Irradiation	1	Epdidymal or vas abnormalities	4
Trauma	1	Phimosis	1
Sexual difficulties	2	Hydrocoele	1

Table 2.1

Summary of clinical findings in 103 patients attending a subfertility clinic with average sperm density under 40 million/ml.

litis while another underwent unilateral orchidectomy following a road accident. Two patients reported psychosexual difficulties. No abnormalities were found on physical examination in half of the patients (Table 2.1). Bilateral (27) and unilateral (7) small testes - under 15 ml volume as measured by Prader's orchidometer, were found in 34 patients. Twelve patients had clinically detectable varicocoeles and four had palpable abnormalities of the epididymis or vas deferens. Phimosis and hydrocoele were each present in one patient.

Thirteen healthy normal subjects who had fathered one or more children within the past five years were also investigated hormonally as controls. Their median age was 31 and range 24-37 years.

2.2.2 Endocrine investigations

In the first 63 patients and the 13 controls, basal samples of LH, FSH and testosterone were obtained at 30 minute intervals for 1½ hours. This was followed by an IV bolus injection of 50 μg GnRH (Gonadorelin, Ayerst) and samples for LH and FSH collected at 30, 60 and 90 minutes afterwards. In the subsequent 40 patients, only single samples of basal LH, FSH and testosterone were obtained.

2.2.3 Analysis of GnRH response

Examination of the LH and FSH profiles after GnRH stimulation (Fig 2.1) suggested that a model of exponential decline might be appropriate, with a relatively slower decline in FSH which might effectively be zero for the duration of the test (i.e. constant stimulated level).

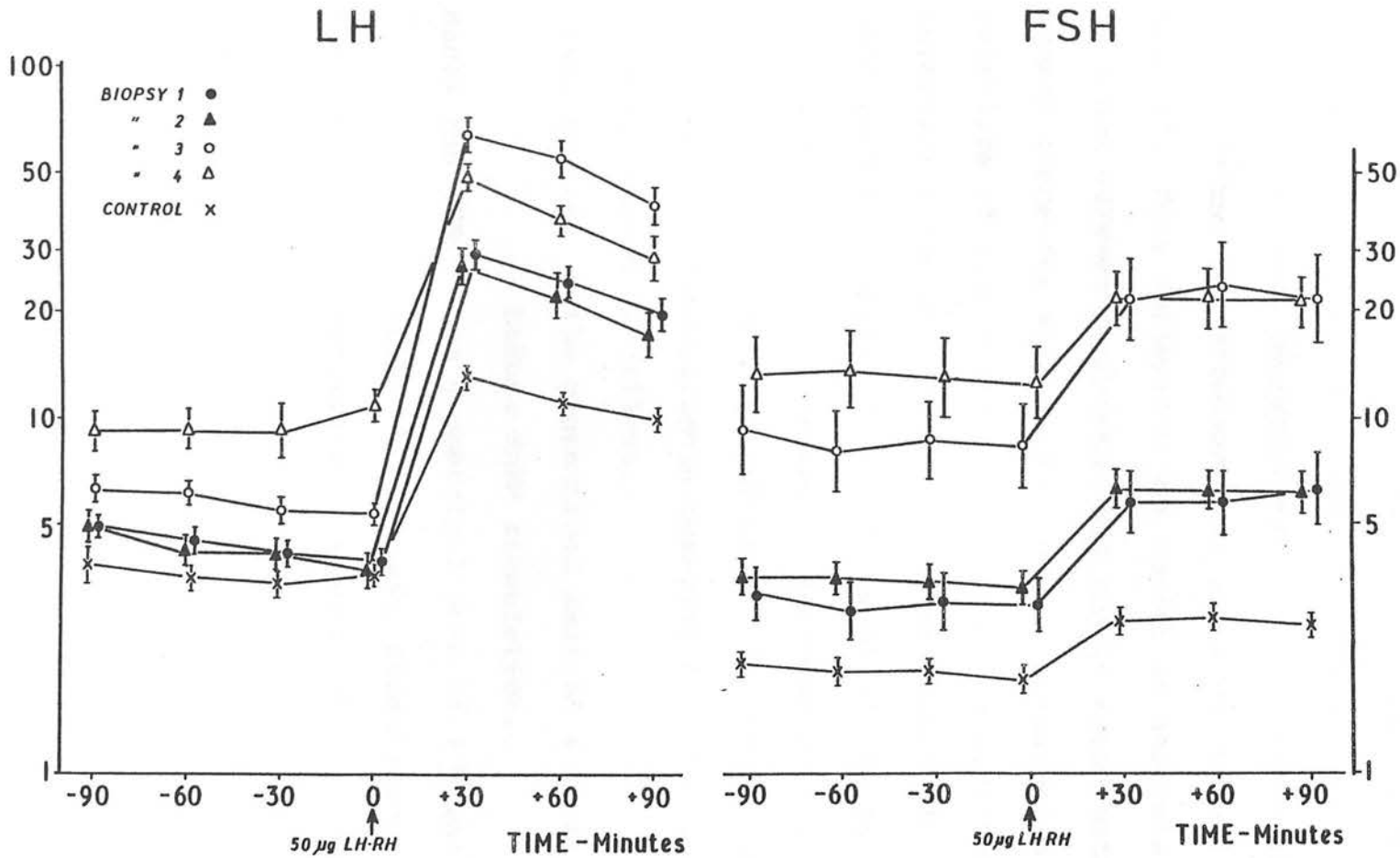


Fig 2.1

Profiles of mean basal gonadotrophins and response to 50 µg of GnRH i.v. in 61 patients divided into the four grades of testicular histology (see text) and 13 normal controls.

All gonadotrophin values were log transformed since both LH and FSH in the one hundred blood donors had log normal distributions. For each individual, a straight line fit to log (stimulated-mean basal) LH and FSH values with time was obtained by least squares. Pooled estimates of the slopes of these straight lines for the whole group were obtained for each gonadotrophin. This assumed that the half-life for the hormones were the same for all subjects studied. This assumption was tested by analysis of variance between individual and common slope models. A common slope for all subjects which corresponded to an LH half-life of 1.36 hours (1.20-1.56 - 95% confidence interval) after GnRH stimulation was confirmed. It also confirmed a constant stimulated level of FSH for each individual, with no increase or decrease between 30 and 90 minutes after GnRH stimulation. In the following analyses, the gonadotrophin measures for each individual were designated as follows:-

Basal LH (LB) - the geometrical mean of 4 basal LH values before GnRH stimulation.

Basal FSH (FB) - the geometrical mean of 4 basal FSH values before GnRH stimulation.

Peak LH (LP) - estimated 30 minute value (minus LB) obtained by fitting a straight line with the common slope to the set of Log (peak-basal) LH readings for each individual.

Peak FSH (FP) - the geometrical mean of the 3 stimulated FSH values minus FB.

2.2.4 Testicular biopsy and histology

Under general anaesthesia, unilateral testicular biopsies in 90 patients and bilateral biopsies in 12 were obtained through small scrotal incisions. One of the latter group had very different histological appearances in the two testes and one patient refused to undergo the procedure after his endocrine investigations. These two patients were excluded from subsequent analyses involving testicular histology. The biopsies were fixed in Bouin's solution and embedded in paraffin. The sections (5 μ thick) were stained with haematoxylin and eosin. Testicular histology was graded according to the proportion of seminiferous tubules showing spermatogenic activity and the degree of such activity within tubules (McIlree et al, 1966; Chandley et al, 1976). Definition of the gradings were as follows:-

Grade 1 - All tubules show active spermatogenesis with production of mature sperms.

Grade 2 - Some or all tubules show depression or arrest of spermatogenesis at various stages or a diminished number of germ cells. No tubules show a complete absence of germ cells.

Grade 3 - Some but not all tubules show a complete absence of germ cells and contain only Sertoli cells.

Grade 4 - Germ cells are absent from all tubules examined, their only content being Sertoli cells.

Thickening of the tunica propria, hyalinization of the tubules, or increase in Leydig cell numbers may occur in Grades 3 and 4.

2.2.5 Semen analysis

Semen analysis was based on the method described in Chandley et al, 1976. The sperm density was estimated by haemocytometer method using an improved Neubauer chamber. The well-mixed seminal fluid was counted in 1:10 or 1:20 dilution depending on an initial estimated count. The diluent consisted of 50 G $NaHCO_3$ and 10 ml of 35% formalin made up to a final volume of 1000 ml with distilled water. All spermatozoa were counted in the large central square of the haemocytometer, which has a multiplication factor of 10,000. For the samples with very low sperm densities, five large squares of the haemocytometer were counted with 2000 being the multiplication factor. Sperm density was expressed as numbers of sperms (in millions) per millilitre of semen. In each patient the mean sperm density from at least two separate samples was used. Although the sperm motility and morphology were analysed, only mean sperm density was used for the present analysis.

2.3 Results

The distribution of testicular histology and sperm density in the 100 patients is presented in Table 2.2. Of the 24 patients with Grades 3 and 4 histology only one had an average sperm density over 5 million/ml. In contrast, the full range of sperm density from azoospermia to 40 million/ml was encountered in the 76 patients with Grades 1 and 2 histology. Alternately, in patients with sperm density over 5 million/ml, all except one had Grade 1 or 2 histology. In those with sperm density under 5 million/ml, any histological grade could be present. It is in this last group that hormone studies may prove to be most helpful.

The relationship between testicular histology and the gonadotrophin and testosterone levels in the first 63 patients who underwent GnRH testing is shown in Table 2.3. Although there was no significant difference in testosterone between any of the patient or control groups, the four gonadotrophin measures (FB, FP, LB and LP) separated the patients into two broad histological groups: Grade 1 with 2 and Grade 3 with 4. This pooling of gonadotrophin measures in terms of similar means and standard deviations is particularly well illustrated by the profiles of FSH responses to GnRH in Fig 2.1.

To determine the best gonadotrophin measure(s) in distinguishing between the two histological groupings (Grades 1 and 2 from Grades 3 and 4), multivariate discriminant analysis (Marriot, 1974) was used. This calculates the generalized distances (Mahalanobis's D)

	Sperm Density						
Testicular Histology	Azoo	1.0 M/ml	1.1-5.0 M/ml	5.1-10.0 M/ml	10.1-20.0 M/ml	20.1-40.0 M/ml	Total
Grade 1	11	2	11	8	15	7	54
Grade 2	2	2	6	4	4	4	22
Grade 3	1	11	2	0	1	0	15
Grade 4	5	3	1	0	0	0	9
Total	19	18	20	12	20	11	100

Table 2.2

The distribution of sperm density and testicular histology in 100 patients. Three patients had been excluded due to inadequate information in semen analyses (1) and testicular histology (2).

Testicular Histology	Number of Patients	Basal FSH U/l	Peak FSH U/l	Basal LH U/l	Peak LH U/l	Testosterone nmol/l
Grade 1	33	3.32 (2.69-4.10)	2.10 (1.49-2.94)	4.39 (3.74-5.15)	18.73 (14.88-23.57)	21.75 (19.87-23.63)
Grade 2	12	2.53 (1.80-3.56)	2.29 (1.30-4.06)	4.14 (3.32-5.16)	16.78 (11.94-23.57)	20.34 (17.62-23.06)
Grade 3	9	9.30 (6.11-14.15)	13.07 (8.17-20.91)	5.42 (4.62-6.36)	39.25 (30.27-50.91)	21.66 (16.68-26.64)
Grade 4	7	13.20 (8.76-19.89)	7.61 (5.58-10.38)	9.58 (7.54-12.18)	25.79 (19.30-34.47)	21.06 (17.11-25.01)
Grade 1 & 2	45	3.10 (2.66-3.61)	2.14 (1.67-2.74)	4.35 (3.89-4.87)	18.17 (15.41-21.44)	21.04 (19.71-22.37)
Grade 3 & 4	16	10.80 (8.09-14.43)	10.28 (7.73-13.67)	6.96 (5.84-8.29)	32.79 (27.04-39.76)	21.36 (18.51-24.21)
Control	13	1.86 (1.59-2.18)	0.61 (0.42-0.90)	3.53 (3.04-4.09)	9.78 (8.51-11.23)	20.18 (17.46-22.94)

Table 2.3

Mean levels and 95% confidence limit in parenthesis of basal and peak (response to 50 µg GnRH IV) gonadotrophins and basal testosterone in the 4 grades of testicular histology (see text) and controls. Patients with testicular histology Grade 1 were pooled with Grade 2 and Grade 3 with Grade 4 since the hormone levels were not significantly different in these subgroups.

which give the differences between the two patient groups and controls in standard deviation units allowing for the correlation between the variables (Table 2.4). Either basal FSH or peak FSH alone could provide the best separation between the two histological groupings and no increase in discrimination was obtained by including further hormonal parameters. The difference in LH between the histological groups could entirely be explained by the correlation between LH and FSH measures ($r = 0.63-0.74$). Basal and peak FSH were highly correlated ($r = 0.75$, $p<0.00001$) in the patient and control groups (Fig 2.2)so that either one of these measures would not improve the discriminant function of the other. Using basal FSH alone with the upper limit of the normal range at 6.7 U/l, elevated values were found in 81% (13/16) of Grades 3 and 4 biopsies and normal values in 91% (41/45) of Grades 1 and 2 biopsies.

The best discrimination between normal controls and the two subfertile patient groups was provided by the LH measures (Table 2.4). Peak LH was the best single parameter distinguishing controls from patients with Grade 1 or 2 histology. The addition of other measures did not significantly improve the discrimination. Although either peak FSH or peak LH alone provided a very clear cut separation between controls and patients with Grade 3 or 4 histology, the best discrimination was given by the combination of basal and peak LH.

The relationship between sperm density and the five hormonal parameters is shown in Table 2.5. Five azoospermic

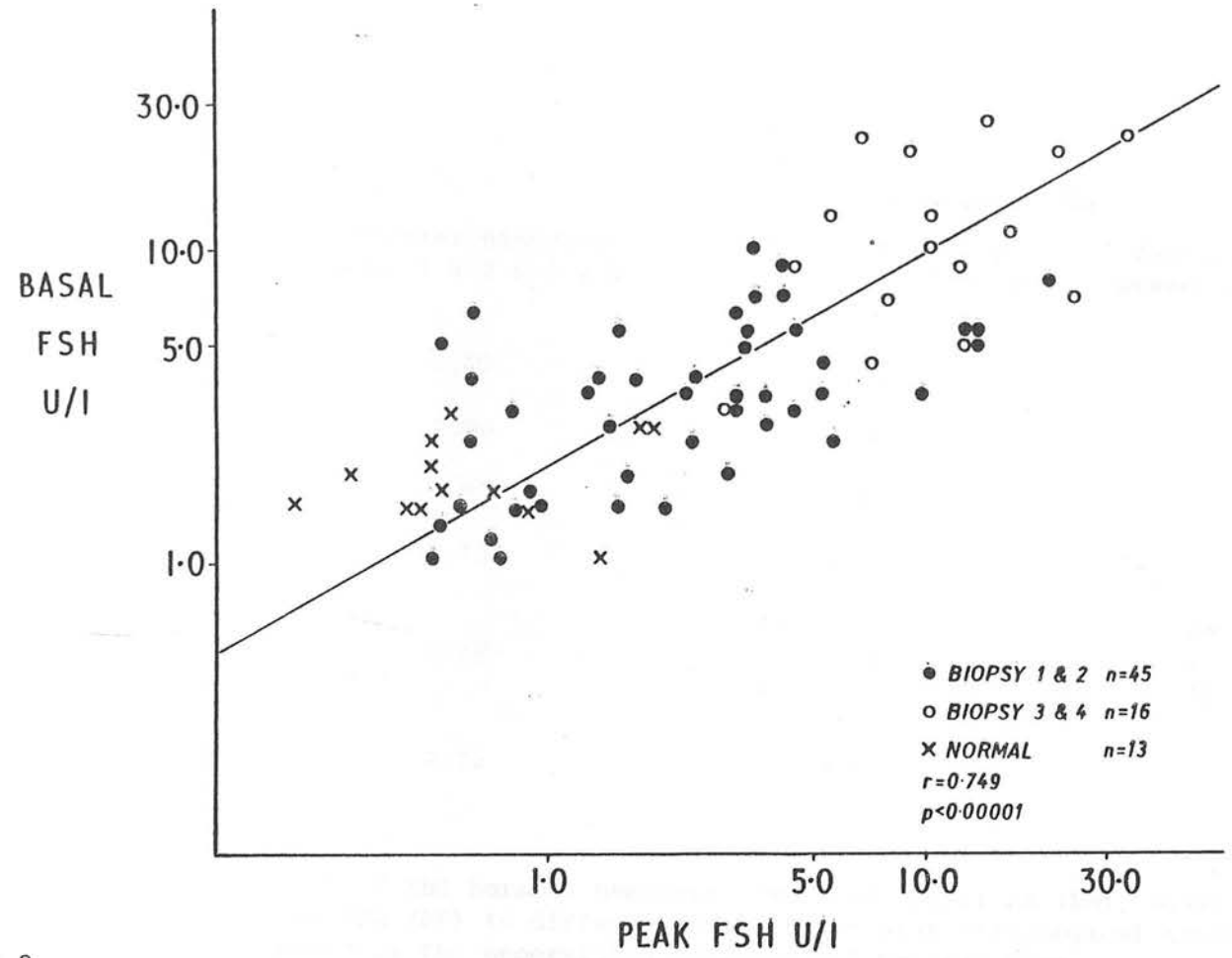


Fig 2.2

Basal and peak FSH (response to 50 μg GnRH i.v.) were highly correlated in the 61 patients and 13 controls.

Hormone Measure	Generalised Distance (D) Testicular Histology Grades 1 & 2 v 3 & 4	Testicular Histology Grades 1 & 2 v Controls	Testicular Histology Grades 3 & 4 v Controls
LB Alone	1.07	0.22	1.88
FB Alone	2.00	0.50	3.31
LP Alone	0.97	1.46	3.89
FP Alone	1.71	1.34	4.31
Best Pair	FB + FP 2.18	LP + FP 1.58	LB + LP 5.36
All 4 Together	2.22	1.60	5.85

Table 2.4

Discriminant functions of the hormone measures (see text) basal LH (LB), basal FSH (FB), peak LH (LP) and peak FSH (FP) in differentiating testicular histological groups and controls as represented by the generalised distnace (D) between them.

Sperm Density	Number of Patients	Basal FSH U/l	Peak FSH U/l	Basal LH U/l	Peak LH U/l	Testosterone nmol/l
Non-obstructive Azoospermia	3	17.82 (6.38-49.83)	7.39 (2.45-22.08)	10.63 (10.11-11.18)	22.12 (9.67-50.61)	22.86 (17.55-28.17)
<1.0 M/ml	13	8.80 (6.07-12.76)	9.96 (6.73-14.73)	5.68 (4.50-7.17)	32.11 (24.88-41.43)	19.61 (16.83-22.39)
1.1-5.0 M/ml	11	4.32 (3.44-5.41)	3.57 (2.00-6.38)	4.24 (3.11-5.76)	22.97 (14.90-35.41)	21.99 (19.37-24.61)
5.1-10.0 M/ml	6	2.69 (1.40-5.17)	2.83 (0.90-8.85)	4.93 (1.25-6.98)	20.81 (14.06-30.79)	20.53 (15.13-25.93)
10.1-20.0 M/l	16	3.18 (2.45-4.12)	2.33 (1.66-3.27)	4.56 (4.18-4.97)	19.69 (14.53-26.68)	22.81 (20.03-25.39)
20.1-40.0 M/l	8	3.40 (2.02-5.73)	1.22 (0.64-2.31)	4.65 (3.07-7.04)	13.46 (9.56-18.94)	19.11 (16.34-21.88)
Obstructive Azoospermia	5	2.62 (1.50-4.60)	1.22 (0.59-2.49)	4.16 (2.45-7.05)	16.18 (11.67-22.43)	21.98 (14.79-29.17)
Normal Controls	13	1.86 (1.59-2.18)	0.61 (0.42-0.90)	3.53 (3.04-4.09)	9.78 (8.51-11.23)	20.20 (17.46-22.94)

Table 2.5

Mean levels and 95% confidence limit in parenthesis of basal and peak (response to 50 μg GnRH IV) gonadotrophins and basal testosterone at different sperm densities. Patients with obstructive azoospermia had Grade 1 or 2 testicular histologies and major abnormalities in the efferent ducts.

patients with normal FSH and Grade 1 or 2 testicular histology were considered to have obstructions in the excurrent ducts and were excluded from this analysis. Both basal and peak FSH showed a significant linear rising trend ($p<0.0001$) with decreasing sperm density (Fig 2.3). Mean basal FSH remained fairly constant until sperm densities fell below 5 million/ml and only exceeded the upper limit of normal basal FSH range (6.7 u/l) with sperm densities under 1 million/ml. Peak FSH showed a linear rising trend over the whole range of sperm densities from 40 million/ml to azoospermia but the magnitude of the increase at each level was smaller than that seen in basal FSH. Neither testosterone, basal LH or peak LH differed significantly with changes in sperm density but the latter two measures did show a weak degree of linearity.

Thus in the analysis of the first 63 patients, basal FSH was the best single hormonal parameter in terms of its relationship to testicular histology and sperm density. The GnRH response did not contribute any additional useful information in these respects. The within-patient coefficient of variation for four basal samples of FSH at 30 minute intervals was so small (10%) that using a single measurement instead of the mean of four did not alter the conclusions of the above analyses significantly.

In the remaining 40 patients, only single samples of basal FSH, basal LH, testosterone and testicular histology were analysed. Discriminant analyses again confirmed

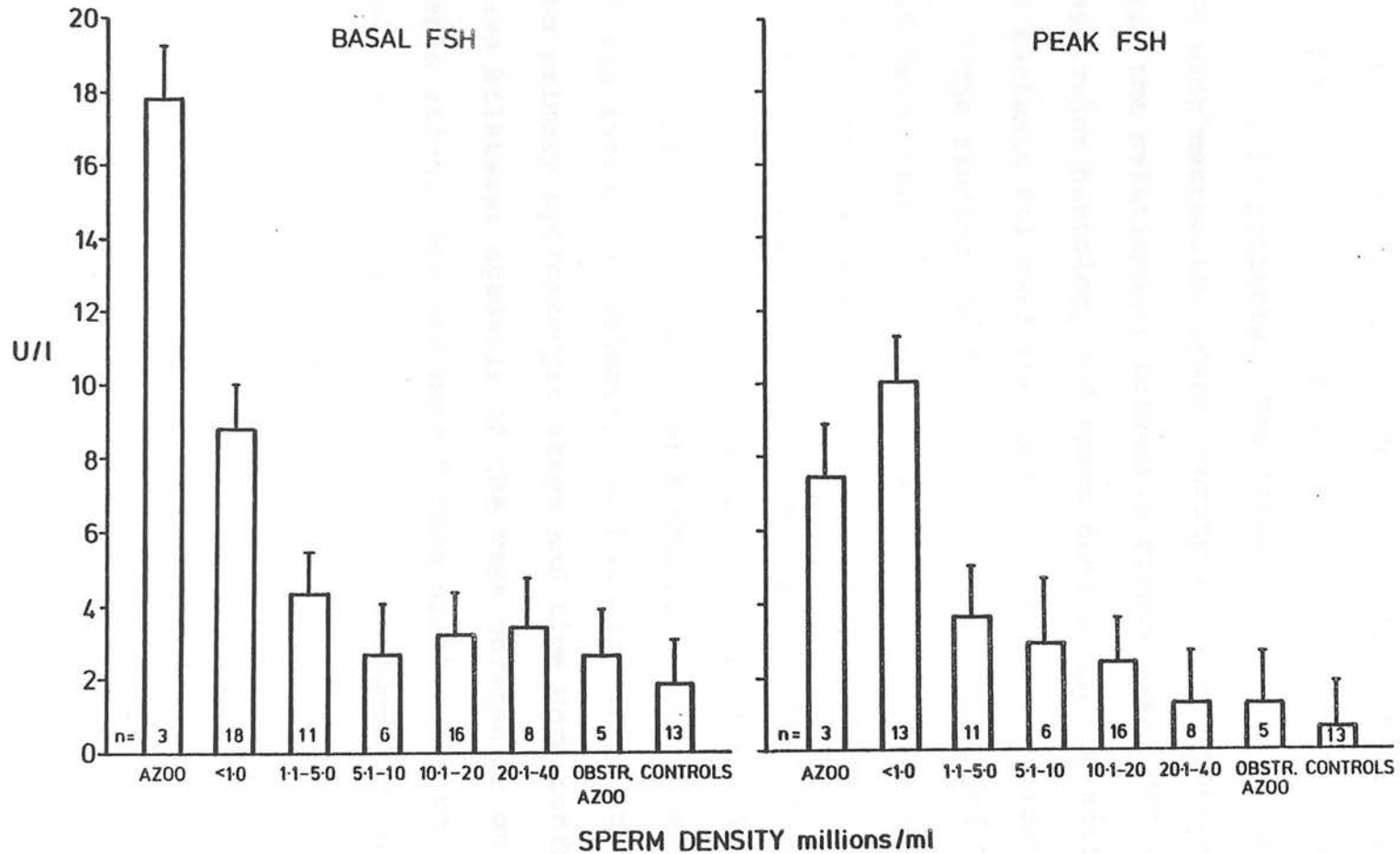


Fig 2.3

Mean (± SEM) levels of basal and peak FSH (response to 50 µg GnRH i.v.) at various sperm densities. Patients with obstructive azoospermia had grade 1 or 2 testicular histologies and major abnormalities of the efferent ducts.

basal FSH to be the best parameters in distinguishing the two histological categories. The addition of basal LH and testosterone did not improve the discriminant function. Elevated basal FSH was found in 75% (4/6) of Grades 3 and 4 histologies and normal FSH in 97% (30/31) of Grades 1 and 2 histologies. This was similar to the findings in the first 63 patients. The linear rising trend in basal FSH with decreasing sperm density was also confirmed. Thus the relationship between a single basal FSH and testicular histology and sperm density in the additional 40 patients followed the same pattern as the first 63.

The findings in 100 patients are summarised in Fig 2.4. and demonstrate the interrelationship between basal FSH, testicular histology and sperm density. In the 19 azoospermic patients, basal FSH clearly differentiated the six with severe germ cell damage (Grades 3 and 4 histologies) from the 13 with Grades 1 and 2 histologies. Of the latter 13 patients, one had maturation arrest at the primary spermatocyte stage and five were confirmed to have bilateral agenesis of the vasa deferentia on scrotal exploration. One had patent vasa bilaterally on vasography and the obstruction was presumed to be in the epididymis. Six patients were not submitted to either scrotal exploration or vasography as they were reluctant to pursue further investigations or treatment. These 13 patients form a separate aetiological group and were excluded from subsequent analyses. In the other 81 patients (oligospermic) normal FSH and Grades 1 and 2 histologies were found in all those with sperm densities

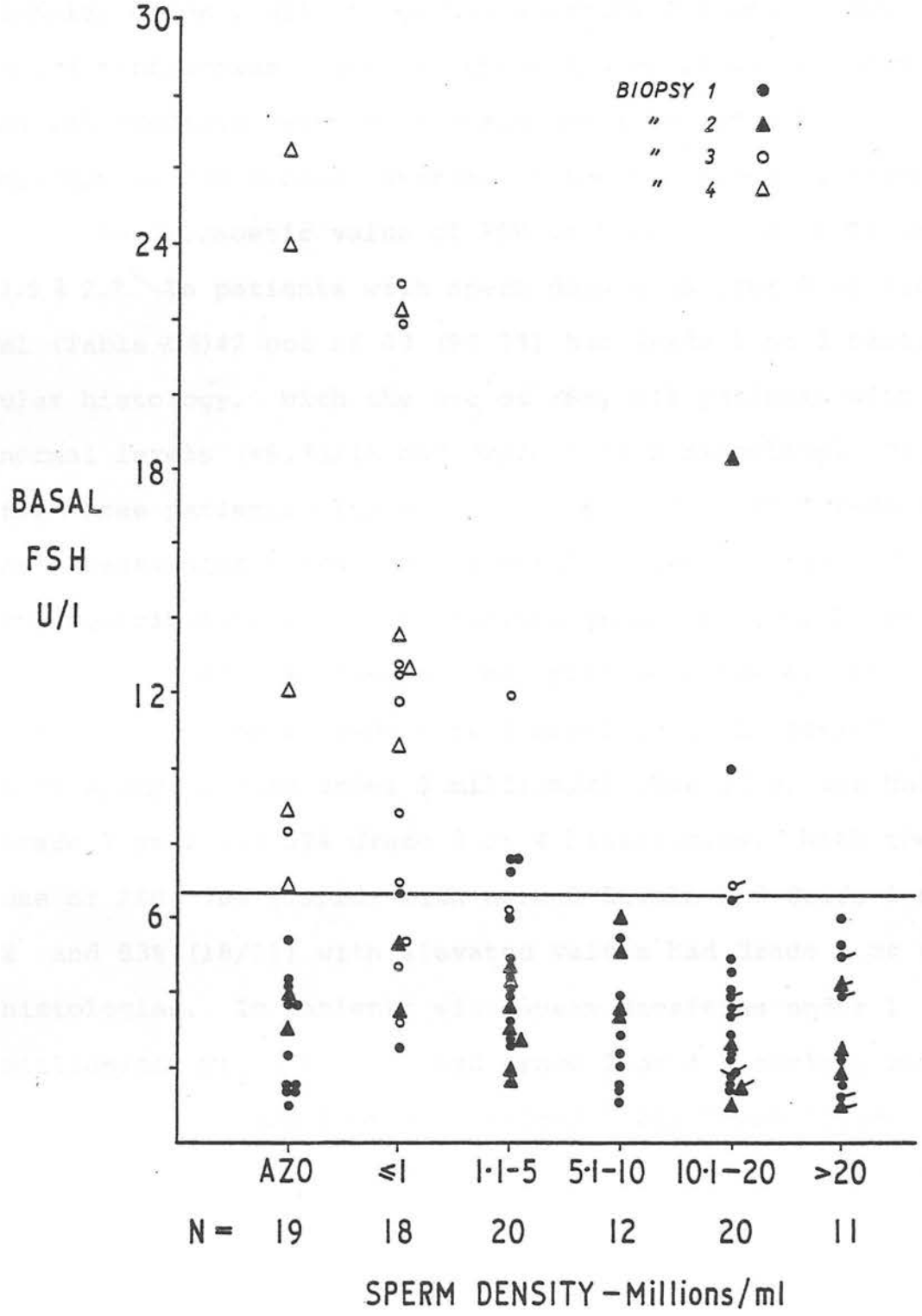


Fig 2.4

Basal FSH, sperm density and testicular histology (grades 1-4, see text) in 100 subfertile men. Horizontal line respresents the upper limit of the normal range (6.7 u/l) for basal FSH in 100 healthy male blood donors. The markers indicate patients who subsequently impregnated their wives with or without treatment.

over 5 million/ml with the exception of three patients. On the other hand, the majority of patients with sperm density under 1 million/ml had elevated FSH and Grades 3 and 4 histologies. Between these two extremes, there was an intermediate group with sperm densities of 1-5 million/ml and greater overlap of the histological groups.

The diagnostic value of FSH is highlighted in Tables 2.6 & 2.7. In patients with sperm densities over 5 million/ml (Table 2.6)42 out of 43 (97.7%) had Grade 1 or 2 testicular histology. With the use of FSH, all patients with normal levels (<6.7u/l) had Grade 1 or 2 histology. Of the three patients with elevated (>6.7 u/l) FSH, Grade 1 or 2 testicular histology was still present in two. Thus the contribution of FSH is limited in patients with sperm densities over 5 million/ml who, with very few exceptions, are likely to have Grade 1 or 2 histology. In patients with sperm density under 5 million/ml (Table 2.6) 48% had Grade 1 or 2 and 52% Grade 3 or 4 histologies. With the use of FSH, 78% (18/23) with normal levels had Grade 1 or 2 and 83% (18/21) with elevated values had Grade 3 or 4 histologies. In patients with sperm densities under 1 million/ml (Table 2.7) 83% had Grade 3 or 4 histology and only 17% had Grade 1 or 2 histology. All these patients with elevated FSH had Grade 3 or 4 histology but in those with normal FSH, both histological groups were found with similar frequencies - 57% Grade 3 or 4 and 43% Grade 1 or 2. Thus, in subfertile patients with sperm densities of under 5 or 1 million/ml, the finding of elevated FSH levels would confirm severe germ cell damage but normal FSH is not helpful.

Sperm Density m/ml	Basal FSH U/l	Testicular Histology 1 & 2	Testicular Histology 3 & 4	Total
>5	-	42 (97.7%)	1 (2.3%)	43
>5	<6.7	40 (100%)	0	40
>5	>6.7	2 (67%)	1 (33%)	3
<5	-	21 (48%)	23 (52%)	44
<5	<6.7	18 (78%)	5 (22%)	23
<5	>6.7	3 (17%)	18 (83%)	21

Table 2.6

Distribution of 87 subfertile patients according to sperm density (over or under 5 million/ml), basal FSH (normal - under 6.7 U/l or elevated - over 6.7 U/l), and testicular histology (Grades 1 and 2 or Grades 3 and 4). Thirteen patients with obstructive azoospermia were excluded.

Sperm Density m/ml	Basal FSH U/l	Testicular Histology 1 & 2	Testicular Histology 3 & 4	Total
>1	-	59 (94%)	4 (6%)	63
>1	<6.7	54 (96%)	2 (4%)	56
>1	<6.7	5 (71%)	2 (29%)	7
<1	-	4 (17%)	20 (83%)	24
<1	<6.7	4 (57%)	3 (43%)	7
<1	<6.7	0	17 (100%)	17

Table 2.7

Distribution of 87 subfertile patients according to sperm density (over or under 1 million/ml), basal FSH (normal - under 6.7 U/l or elevated - over 6.7 U/l) and testicular histology (Grades 1 and 2 or Grades 3 and 4). Thirteen patients with obstructive azoospermia were excluded.

2.4 Discussion

In the assessment of male infertility, two key issues are central to the management of the patient, the fertility potential of the individual and the degree of damage to the seminiferous tubules. The present findings are mainly, though not exclusively, concerned with the latter aspect. Although conventional semen analysis can provide some indication of changes in the seminiferous epithelium (Johnsen, 1970a; Kjessler & Wide, 1973; Aafjes et al, 1977) the degree of abnormality in spermatogenesis is not always accurately reflected by this investigation (for example, obstruction in the excurrent ducts and spermatogenetic arrest). Biopsy of the testes has therefore been employed to enable seminiferous tubular histology to be assessed directly. In common with other feedback control systems, dysgenesis or damage to the seminiferous tubules will result in increased secretion of gonadotrophins, especially FSH, due to the reduction or loss of negative feedback. This reciprocal relationship of FSH to both sperm density (Johnsen, 1970b; Kjessler & Wide, 1973; Mauss & Börsch, 1973; Hunter et al, 1974; Christiansen, 1975; Aafjes et al, 1977) and testicular cytology (Johnsen, 1970b; Rosen & Weintraub, 1972; Kjessler & Wide, 1973; Mauss & Börsch, 1973; Hunter et al, 1974; Christiansen, 1975; Aafjes et al, 1977) may thus improve the correlation between these two parameters of seminiferous tubule function and obviate the need for testicular biopsy.

Our findings showed that in terms of the inverse

relationships with sperm density and the degree of germ cell loss demonstrated by testicular histology, basal FSH, amongst the various combinations of hormone measures, gave the greatest discriminant value. This confirmed the findings in previous studies (Johnsen, 1970b; Rosen & Weintraub, 1972; Kjessler & Wide, 1973; Mauss & Börsch, 1973; de Kretser et al, 1974; Hunter et al, 1974; Christiansen, 1975; Aafjes et al, 1977). The variation of basal FSH at intervals of 30 minutes was small and single estimations were representative. The additional measurements of basal LH, testosterone and the GnRH response did not contribute further to the discriminant function of basal FSH alone.

In our analysis of testicular histology, endocrine studies clearly differentiated between patients with complete absence of germ cells in some or all tubules (Grade 3 or 4) from those in which germ cells were present in normal or reduced numbers. This is in agreement with the findings of de Kretser et al (1972), Franchimont et al (1972) and Hunter et al (1974), who used similar qualitative analyses of testicular histology. De Kretser et al (1974), using quantitative methods, confirmed the inverse relationship between FSH and the number of germ cells in seminiferous tubular cross-sections. However it is doubtful whether such time-consuming quantitative analyses of testicular histology are more useful than the simpler qualitative classification used in this study in the clinical assessment of infertility.

The finding that the FSH response to GnRH stimulation

was highly correlated with basal FSH confirms the previous studies (Isurgi et al, 1973; Mecklenberg & Sherins, 1974; Franchimont, 1975; Roulier et al, 1976). Lipschulz et al (1977) however, demonstrated an exaggerated FSH response to 250 μg of GnRH in spite of normal basal levels in oligospermic patients with sperm density under 10 million/ml. This discrepancy may be due to the difference in GnRH dose employed and the different criteria for selection of oligospermic patients (under 40 million/ml in the present study). A further explanation lay in the different relationship of sperm density to basal and peak FSH. Basal FSH remained fairly constant and only increased outwith the normal range when sperm density fell below 5 million/ml. A similar finding has been reported by Mauss & Börsch (1973) although the critical level of sperm density was 10 million/ml. The FSH response to GnRH (peak FSH) however showed a linear rising trend over the entire range of sperm density from 40 million to azoospermia. Thus patients with sperm density around 5-10 million/ml may have basal FSH within the normal range but exaggerated FSH response to GnRH - the group of patients that Lipschultz et al (1977) selected for investigation. However, even in that study, basal FSH in the oligospermic patients were significantly elevated compared to controls, though the individual levels remained within the normal range. Furthermore, the magnitude of the GnRH response was proportional to basal FSH in all patient groups and controls. Their findings are therefore entirely compatible with those of

the present study.

The observation that the GnRH response was superior to basal FSH in distinguishing between normal fertile controls and the subfertile patients implies that the former measure may be an index of fertility potential - the lower the GnRH response, the higher the fertility. The small numbers of fertile controls and their lack of semen analysis data in this study do not permit any firm conclusions but further studies are indicated.

The elevated and peak LH found in patients with Grades 3 and 4 histology confirmed the reports of de Kretser et al (1972), Hunter et al (1974) and Christiansen (1975). However, the testosterone levels in our patients were normal and a tendency for lower testosterone in patients with severe germ cell damage was not demonstrated. de Kretser et al (1972), however, found lower or subnormal levels of testosterone and impaired response to HCG stimulation (de Kretser et al, 1975b) in patients with seminiferous tubular hyalinization of Sertoli cell only syndrome although there was no clinical evidence of androgen deficiency. This apparent discrepancy is due to the exclusion of patients with chromosomal abnormalities (Klinefelter's and variants) and the smaller proportion of patients with more severe seminiferous tubular damage in the present study. The elevated LH with or without low testosterone may be the result of compensated Leydig cell failure presumably caused by the same aetiological factors which affected the seminiferous tubular compartment. Recent evidence from in vitro studies (Rodriguez-Rigau et al,

1978; Weiss et al, 1978) confirmed abnormalities in Leydig cell functions in infertile men but the possibility of Leydig cell failure being a consequence of germ cell damage cannot be excluded. It is interesting to note that low plasma testosterone found in subfertile patients with varicoeles reverted to normal after surgical ligation of the internal spermatic vein (Comhaire & Vermeulen, 1975).

Summarizing the clinical implications of the foregoing considerations: the most informative endocrine investigation in the management of severely oligospermic and azoospermic patients is a single estimation of basal FSH, raised levels of which are indicative of severe and probably irreversible germ cell damage. The response to GnRH stimulation confers very little additional information and is not indicated in routine management of male infertility. Measurement of LH and testosterone has limited value in assessing the degree of germ cell damage in patients without clinical evidence of androgen deficiency, although they may be useful for detection of androgen insensitivity (Aiman et al, 1979).

Having established the role of endocrine investigations it will be necessary to reconsider the indications for testicular biopsy in the assessment of male infertility. From the results of this study and that of Pryor et al (1978), it is clear that in azoospermic patients, FSH can differentiate those with efferent duct obstruction and maturation arrest from those with germ cell depletion (Fig 2.4). The latter group with elevated

FSH will not require further investigation and can be advised to consider the alternatives of adoption or artificial insemination by donor at an early stage. For the azoospermic patients with normal FSH, scrotal exploration, vasogram and testicular biopsy should be performed in order to demonstrate the obstruction and prior to epididymo-vasostomy. The importance of testicular biopsy, especially when the epididymides appear normal on exploration, should be emphasised. This enables germ cell maturation arrest to be recognized as these patients are not suitable for epididymo-vasostomy. Baker et al (1976) found that 52% of patients with germ cell arrest had elevated FSH levels. The classification of testicular histology in our study did not identify germ cell arrest as a separate entity so that this finding could not be confirmed. Patients with agenesis of the vasa deferentia can be diagnosed on clinical examination and confirmed by the absence of fructose in the semen without resorting to scrotal exploration or testicular biopsy (Amelar & Hotchkiss, 1963). For oligospermic patients with sperm density under 5 million/ml, elevated FSH has virtually the same implications as in azoospermic patients. If FSH is normal, testicular histology cannot be reliably predicted and biopsy is required if clinical decisions are made on the basis of histological changes in the seminiferous epithelium. The value of vasography in this group of patients is at present unknown. For oligospermic patients with sperm density over 5 million/ml, neither testicular biopsy nor

FSH estimation is required since the vast majority have normal levels of FSH and Grade 1 or 2 histology (Fig 2.4).

There were a number of patients that did not conform with predictions in this diagnostic scheme: five patients with elevated FSH but Grades 1 and 2 histology and five with normal FSH but Grades 3 and 4 histology. Valid explanations may be presented for these apparent anomalies. Unilateral biopsies were performed in all except twelve cases. Where the two testes in a patient were of dissimilar size or consistency, the one considered more normal clinically was biopsied. Although it is generally held that testicular size is a good index of spermatogenesis in adults (Rifka & Sherins, 1978) several studies have emphasized that this may not be entirely reliable (Meinhard et al, 1973; Pryor et al, 1976; Guay et al, 1977). In the present context, the five patients with normal FSH and Grade 3 or 4 histology may have more active spermatogenesis in the unbiopsied contra-lateral testis providing an adequate feedback to suppress FSH. The alternative explanation may be that of pituitary failure resulting in inappropriately low FSH. Two out of these five patients subsequently underwent full pituitary function testing, the results of which were normal. The finding that all five patients had normal LH and testosterone and a normal or exaggerated response to GnRH makes the possibility of pituitary failure very unlikely. Nevertheless, this may be the group of patients in which gonadotrophin therapy should be considered.

One interesting finding in this study is that

amongst the six patients with unilateral atrophic or absent testes, three had elevated basal levels in spite of relatively good sperm production of over 15 million/ml. In a further two patients in this group the FSH response to GnRH was greatly exaggerated even though basal FSH was normal. This may be interpreted as a state of compensated spermatogenic failure with the hypothalamic-pituitary unit being readjusted to a higher set point in response to the lower level of testicular feedback due to unilateral testicular atrophy. Whether compensatory hypertrophy occurs in the contralateral testis in these patients could not be assessed from the present study. Laron et al (1975) postulated that compensatory hypertrophy occurs in the normal contralateral testis in patients with unilateral cryptorchidism although the seminiferous tubular diameter remains unchanged. They also found FSH to be elevated in these patients with unilateral cryptorchidism, a finding which was subsequently confirmed by Werder et al (1976) and Lipschultz et al (1976). In our patients, however, unilateral testicular atrophy resulted not only from cryptorchidism but also from varicocoele, mumps orchitis, orchidectomy and radiation. The clinical significance of our finding is that in the presence of obvious unilateral atrophic or absent testes from whatever aetiology, elevated FSH levels are compatible with relatively good sperm production or even fertility and do not necessarily indicate severe germ cell failure.

The finding of selective or preferential hypersecretion of FSH associated with failure of spermato-

genesis in man is frequently cited as evidence supporting the negative feedback role of the seminiferous tubular hormone - inhibin (Section A, Chapter 12). Yet, the FSH concentrations even in those patients with grade 4 testicular histology (Sertoli cell only), were considerably lower than those found in castrate or agonadal men. This finding confirmed the experimental results of Morris & Jackson (1978) and suggests that the seminiferous tubules are only partially responsible for the negative feedback control of FSH. Although it may be argued that the Sertoli cells may retain their inhibin-producing capacity even in the face of germ cell aplasia, the alternative explanation that testosterone and/or oestradiol are important in FSH negative feedback control is an attractive one. Total circulating testosterone concentrations in infertile men in this study are normal. However, sex hormone binding globulin, unbound testosterone and oestradiol were not studied. A physiological role for testicular oestrogens has not been defined and this remains a promising area for investigation. If either testosterone or oestradiol in physiological concentrations can suppress FSH, it begs the question of whether FSH might also play a role in the regulation of testicular steroidogenesis. In hypo-physectomized animals, FSH can potentiate the trophic effect of LH (Bartke, 1978) although the mechanism and the site of action are unknown. These observations pose the interesting possibility that Sertoli and Leydig cells interact closely in the physiological regulation of

testicular steroidogenesis and spermatogenesis. Thus the endocrine and exocrine functions of the testis, though occurring in different anatomical compartments, are functionally integrated possibly by local intra-testicular regulatory mechanisms.

CHAPTER 3

OESTROGENS IN MALE INFERTILITY

3.1 Introduction

In the majority of cases of male infertility, the underlying aetiology and pathogenesis of defective spermatogenesis remains unknown. With the realization that androgens are essential for spermatogenesis (Steinberger, 1971; Steinberger et al, 1978) more attention has been focused on Leydig cell function in infertile males. Testosterone in infertile men has been found to be lower than normal (Rosen & Weintraub, 1971; de Kretser et al, 1972; Aloysio et al, 1974; Purvis et al, 1975; Nieschlag et al, 1978) although this was not confirmed by others (Ruder et al, 1974; Lawrence & Swyer, 1974; Nankin et al, 1977). In vitro studies of testicular biopsy tissue from oligospermic men showed abnormalities in testicular steroidogenesis (Rodriguez-Rigau et al, 1978; Oshima et al, 1977) but, again, this was not substantiated (Nieschlag et al, 1979). There was more general agreement in the finding of elevated LH levels (de Kretser et al, 1972; Hunter et al, 1974; Christiansen, 1975) which may be compatible with primary Leydig cell failure. Furthermore, the hCG response was subnormal in some severely oligospermic men (de Kretser et al, 1975b). The weight of available evidence would support the presence

of Leydig cell dysfunction in men with primary defects of spermatogenesis. The mechanism underlying this observation is at present unknown.

In man, the normal testis secretes oestrogens (Kelch et al, 1972; Baird et al, 1973; Weinstein et al, 1974) although their physiological function is unclear. Dufau et al (1978) recently suggested that oestrogens may have an intratesticular role in the local control of Leydig cell function. The ability of FSH to stimulate the formation of oestradiol from testosterone and androstenedione in rat Sertoli cells in vitro was demonstrated by Dorrington and Armstrong (1975). Oestrogens may therefore be important in the pathogenesis of Leydig cell dysfunction in infertile men with elevated FSH.

The nature of the negative feedback control of FSH by the testes is controversial. FSH inhibiting activity is known to reside in the water-soluble rather than the organic extracts of the testes (McCullagh, 1932). However, oestrogens have also been shown to preferentially suppress FSH secretion (Kulin & Reiter, 1972; Sherins & Loriaux, 1973; Franchimont et al, 1975). The most abundant circulating oestrogen in adult males is a water-soluble oestrogen conjugate, oestrone sulphate; yet no information is available on this steroid in men with elevated FSH resulting from seminiferous tubule damage.

The aim of this study is to investigate the circulating levels of oestrogens in infertile men with pathologically elevated FSH. Oestrone sulphate, oestrone and oestradiol are quantitated in peripheral plasma to

provide comprehensive indices of the oestrogenic status in men with primary disorders of seminiferous tubules.

3.2 Patients and methods

Peripheral venous blood samples were obtained from twenty patients attending the Male Subfertility Clinic, Royal Infirmary, Edinburgh. These patients were selected by the finding of one or more elevated plasma FSH level (normal male range 1.6-6.7 U/l) in association with repeated azoospermia or oligospermia (sperm density under 10 million/ml). Thirteen oligospermic patients had a mean sperm density of 3.1± 2.3, range 0.25-8.5 million/ml. Seven patients were azoospermic. The mean age of this infertile group (n=20) was 28.9 ± 4.3 years. All patients had a normal 46 XY karyotype and normally developed secondary sexual characteristics. Details of the clinical findings are summarized in Table 3.1.

The control group was represented by thirteen men whose wives had delivered normal infants within the previous 4.6 ± 4 months in the Simpson Memorial Maternity Pavilion, Edinburgh. No semen analysis was performed in this group. Blood samples were also obtained from eight male partners of infertile couples attending the Gynaecological Infertility Clinic, Royal Infirmary, Edinburgh; they were considered fertile by virtue of repeatedly normal semen analyses (mean sperm density - 181.5 ± 84.2, range 86-327 million/ml) and the absence of any abnormalities in the history or examination. The mean age of the normal fertile control groups (n=21) was

History		Examination	
Mumps orchitis	- 1	L. varicocoele	- 2
R. Inguinal herniorraphy	- 1	R. undescended testis	- 1
Bilateral orchidopexy	- 3	Small atrophic testes	
		- unilateral	- 1
Unilateral testicular torsion	- 1	- bilateral	- 7

Table 3.1

Clinical findings in 20 infertile men*

*Some patients had more than one of the above abnormalities. One azoospermic and five oligospermic patients did not have any abnormalities in the history or examination.

31.8 ± 4.1 years.

Statistical analyses were performed using Student's single-tailed t test.

3.3 Results

The mean peripheral plasma concentrations of oestrone sulphate, oestrone and oestradiol in infertile males with elevated FSH were significantly higher than that in normal controls. Plasma LH was elevated to a lesser extent than FSH, but testosterone remained indistinguishable from normal (Fig 3.1).

In both azoospermic and oligospermic subgroups, the oestrogens and gonadotrophins remained significantly elevated from normal (Table 3.2). Comparison between azoospermic and oligospermic patients showed no difference with respect to the three oestrogens and testosterone. LH and FSH, however, were higher in the azoospermic group. Mean SHBG binding capacity was significantly higher in eight oligospermic patients than controls but the small number of azoospermic patients did not show any difference from either normal or oligospermic men (Table 3.2). Combined together as one infertile group, SHBG binding capacity was significantly elevated from normal (3.35 ± 0.82 vs $2.76 \pm 0.89 \times 10^8$ M/l, $p<0.05$, not shown in Figure 3.1).

A significant but weak positive correlation was obtained between FSH and oestrone ($r = 0.3946$), oestrone sulphate ($r = 0.3457$) and oestradiol ($r = 0.3221$) when the patient and control groups were combined. Correlations

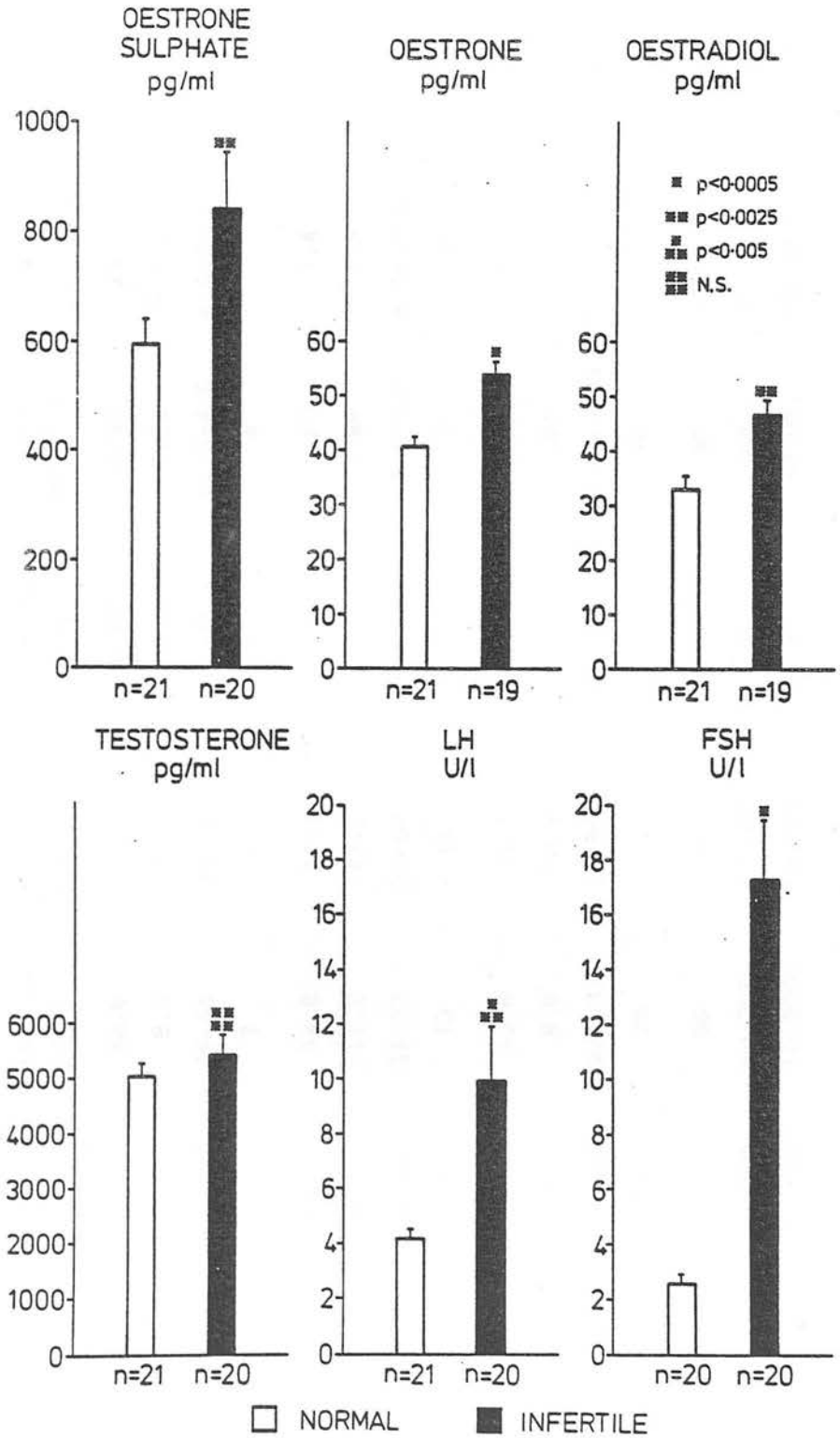


Fig 3.1

Comparisons of the mean (± SEM) plasma concentrations of oestrone sulphate ($p<0.0025$), oestrone ($P<0.0005$), oestradiol ($P<0.0025$), testosterone (N.S.), LH ($P<0.005$) and FSH ($P<0.0005$) between normal and infertile men.

Study Groups		Oestrone Sulphate pg/ml	Oestrone pg/ml	Oestradiol pg/ml	Testosterone pg/ml	SHBG $x10^{-8}$ M/l	LH U/l	FSH U/l
Azoospermic (A)	Mean	829	52.6	50.3	5328	2.96	14.4	27.2
	SD	225	8.6	12.1	1298	0.63	1.4	10.4
	Range	539-1225	36-64	40-76	3974-8024	2.37-4.0	4.4-46.4	12.7-42.7
	N	7	7	6	7	4	7	7
Oligospermic (O)	Mean	851	54.8	44.8	5492	3.60	7.4	12.0
	SD	237	11.2	12.5	1707	0.88	1.5	33
	Range	422-1423	31-77	21-67	2765-8025	2.7-5.0	5.3-11.1	7.2-20.7
	N	13	13	13	13	8	13	13
Normal (N)	Mean	593	40.6	33.1	5046	2.76	4.2	2.6
	SD	220	8.8	10.9	1102	0.89	1.6	1.4
	Range	369-1230	22-58	17-59	3457-7160	1.20-4.74	1.9-8.3	1.0-5.5
	N	21	20	21	21	16	21	20
Statistical Difference P	A v O	NS	NS	NS	NS	NS	<0.05	<0.0005
	A v N	<0.025	<0.005	<0.005	NS	NS	<0.0025	<0.0005
	O v N	<0.0025	<0.0005	<0.005	NS	<0.05	<0.0005	<0.0005

Table 3.2

Comparisons of mean plasma levels of oestrone sulphate, oestrone, oestradiol, SHBG, LH and FSH in azoospermic, oligospermic patients and normal controls. NS = not significant $P \geq 0.05$.

between LH and oestrone sulphate (r = 0.3686), LH and FSH (r = 0.6278) were also present.

Although the mean absolute levels of all three oestrogens were higher in the infertile group, the ratios of oestrone sulphate to oestrone (15.6:1), oestrone sulphate to oestradiol (18.1:1) and oestrone to oestradiol (1.2:1) have remained similar to that in the normal controls - 14.6:1, 17.9:1 and 1.2:1 respectively.

3.4 Discussion

This is the first study describing increased concentrations of plasma oestrone sulphate, oestrone and oestradiol, in infertile men with pathologically raised levels of FSH. It contrasts with the studies of Wieland et al (1974) and Nankin et al (1977) who found normal concentrations of oestradiol in a group of patients with similar abnormalities. Purvis et al (1975) demonstrated normal plasma oestradiol but low oestrone concentrations in ten azoospermic men with elevated FSH levels. Nieschlag et al (1978), however, described a tendency towards higher plasma oestradiol and lower testosterone concentrations with decreasing sperm density in infertile men.

In normal men, oestradiol and oestrone are predominantly derived from peripheral conversion of androgen precursors (Baird et al, 1969), while testicular secretion contributes a small but significant proportion of these androgens (Kelch et al, 1972; Baird et al, 1973; Weinstein et al, 1974). Based on data from steady state

dynamic studies, it has been suggested that oestrone sulphate is almost entirely derived from peripheral conversion of oestrone and oestradiol (Longcope, 1972; Ruder et al, 1972). In the present study, despite increased mean plasma levels of all three oestrogens in infertile men, the ratios between their concentrations were not different from normal. This implies that the dynamics of oestrogen production in infertile men may be qualitatively similar to normal. Thus the excessive oestrogen production in infertile men could have arisen from increased testicular secretion of oestradiol and oestrone and/or enhanced peripheral conversion of androgen precursors.

In infertile men, basal testosterone and androstenedione concentrations and the response to hCG stimulation are either normal or low (de Kretser et al, 1972 & 1975b; Purvis et al, 1975; Nieschlag et al, 1978). There is therefore no evidence to support an increased production of androgen precursors for aromatization in patients with oligo or azoospermia. Normal rates of peripheral aromatization of testosterone and androstenedione in hypergonadotrophic males with or without chromosomal abnormalities were reported by Sitteri & MacDonald (1973); they suggested that increased testicular secretion of oestradiol and oestrone could account for the observed oestrogen excess in these patients. This is supported by the demonstration of increased testicular aromatase activity in patients with Klinefelter's syndrome (Sharma et al, 1971). Raised testicular oestradiol concentration

(Damber & Bergh, 1980) and oestradiol production in response to hCG stimulation (de Kretser et al, 1979) have been demonstrated in the experimentally-induced cryptorchid rat testes. Taken together, the available evidence suggests that increased testicular secretion is the more likely mechanism underlying the excessive oestrogen production in infertile men.

The observation that all three oestrogens were positively correlated with FSH suggests that elevated FSH may be important in stimulating increased testicular oestrogen secretion in infertile men. This is supported by the finding that rat Sertoli cells in culture can aromatize testosterone and androstenedione to oestradiol when incubated with FSH (Dorrington & Armstrong, 1975; Rommerts et al, 1978 & 1979). This FSH-stimulated aromatase activity is maximal in Sertoli cells from prepubertal rats and declined as the animals matured (Armstrong & Dorrington, 1977). The response to FSH was also greater in Sertoli cells after five days compared to one day culture. This was associated with a decrease in the number of contaminating cells including germ cells (Dorrington & Armstrong, 1979). One interpretation of these observations is that germ cells may exert an inhibitory effect on the ability of Sertoli cells to synthesize oestrogens in response to FSH stimulation. Thus the present findings of increased oestrogen production associated with high FSH and germ cell depletion in infertile men are in accordance with the data from the in vitro animal experiments.

A direct inhibitory action of oestrogens on Leydig cell function independent of the systemic suppression of gonadotrophins has been demonstrated by a number of clinical and experimental studies (Baker et al, 1973; Chen et al, 1977; Jones et al, 1978; Hsueh et al, 1978; Van Beurden et al, 1978). The direct binding of labelled oestradiol to cytoplasmic and nuclear binding sites in rat testicular interstitial cells has been demonstrated (Brinkman et al, 1972; Mulder et al, 1974). The mechanism of the direct inhibitory action is probably via suppression of specific enzyme steps (17-20 lyase and 17α hydroxylase) in the testosterone steroidogenic pathway (Samuels et al, 1964 & 1969; Hsueh et al, 1978; Kalla et al, 1980; Brinkman et al, 1980) although a reduction of Leydig cell LH receptor sites may also contribute (Saez et al, 1978; Huhtaniemi et al, 1980). Leydig cell functions could therefore be impaired subsequent upon a primary abnormality of spermatogenesis, increased secretion of FSH and testicular overproduction of oestrogen. This may account for the presence of Leydig cell dysfunction observed in infertile men (Rosen & Weintraub, 1971; de Kretser et al, 1972; Aloysio et al, 1974; Purvis et al, 1975; Oshima et al, 1977; Rodriguez-Rigau et al, 1978; Nieschlag et al, 1978) and in animals with experimentally induced seminiferous tubular damage (Damber et al, 1978; Rich et al, 1979; Kerr et al, 1979; de Kretser et al, 1979; Main & Setchell, 1980). If the stimulus to excessive oestrogen production is sufficiently potent and persistent, testicular oestrogen

secretion into the circulation may also be increased. The consequent diminished supply of androgens to the Sertoli cells may cause a reduction in androgen binding protein production and a fall in intratubular androgens available for the maintenance of spermatogenesis. This hypothetical series of events may form a vicious circle which aggravates or perpetuates the initial insult to the seminiferous epithelium (de Kretser, 1979).

It is noteworthy that concurrent with the rise in plasma oestrogens in infertile men with elevated FSH, SHBG binding capacity was also significantly increased. As excessive endogenous or exogenous oestrogen is well-known for increasing SHBG concentration (Pearlman et al, 1967; Migeon et al, 1968; Vermeulen et al, 1969), this observation provides further supportive evidence for the principle findings in the present study. It is of further interest that the total plasma testosterone concentration in the infertile men was not significantly different from normal in spite of increased circulating LH and SHBG binding capacity. This implies that there is a significant fall in testosterone secretion and unbound testosterone concentration, and confirms the presence of Leydig cell dysfunction in infertile men in this study despite the normal total plasma testosterone concentrations.

That both FSH and to a lesser extent LH remain elevated in the infertile patients also implies that the rise in oestrogen is modest in magnitude or alternatively, that oestradiol plays a minor role in the feedback control of gonadotrophins. While compelling evidence has been

accumulated in recent years supporting the existence of inhibin (Baker et al, 1976; Franchimont et al, 1979), its physiological role in humans in relation to gonadal steroids remains unresolved. Several experimental studies had demonstrated that testosterone, with or without oestradiol, provides the major negative feedback signal for FSH as well as LH secretion (Resko et al, 1977; Plant et al, 1978; Morris & Jackson, 1978; Main et al, 1980a). There is evidence also that oestrogens alone may preferentially suppress FSH (Kulin & Reiter, 1972; Sherins & Loriaux, 1973; Santen, 1977; Sawin et al, 1978; Lacroix et al, 1979) and hence act synergistically with inhibin and/or testosterone (Resko et al, 1977). Since inhibin-like activity resides in the aqueous extracts of testes (McCullagh, 1932), it is interesting to consider the possible role of oestrone sulphate, a water-soluble oestrogen conjugate, in the negative feedback control of FSH. Our results however demonstrated a positive rather than a negative correlation between oestrone sulphate (and the unconjugated oestrogens) and FSH. It seems unlikely therefore that oestrogens have a major physiological role in the feedback control of FSH secretion in man.

The diagnostic value of oestrogen measurement in the assessment of male infertility is probably limited. Other hormonal indices of seminiferous tubule damage are probably more helpful although it must be emphasized that no large scale study has examined the true incidence or the relevance of oestrogen abnormalities in male

infertility. The need remains however to define the exact aetiological role of oestrogens in the pathogenesis of seminiferous tubule disorders. Further studies with in vitro techniques and the direct testicular secretion of these hormones are indicated.

CHAPTER 4

TESTICULAR OESTROGEN SECRETION

4.1 Introduction

Testicular secretion of oestrogen into the spermatic vein was first demonstrated in the perfused stallion testis (Savard & Goldzieher, 1960). The direct secretion of oestrone and oestradiol by the human testis was inferred from the finding of higher concentrations of these oestrogens in spermatic compared to peripheral venous blood (Kelch et al, 1972; Baird et al, 1973; Weinstein et al, 1974). From these studies, it was estimated that testicular secretion contributed approximately one quarter of the total blood production of oestradiol but a somewhat smaller proportion in the case of oestrone. As there is evidence of increased oestrogen production in men with elevated FSH (Chapter 3), the direct and simultaneous measurement of these steroids in spermatic and peripheral venous plasma may clarify the extent of the contribution of testicular secretion to the circulating oestrogen excess in men with disorders of the seminiferous tubules.

It has been suggested that oestrone sulphate, the most abundant oestrogen in the peripheral circulation (Brown & Smyth, 1971), may also be secreted from the human testis (Hembree et al, 1969; Baird et al, 1968) as is the case in the stallion (Raeside, 1969). However, isotope dilution studies have indicated that the entire blood production of oestrone sulphate can be accounted

for by peripheral conversion of unconjugated oestrone and oestradiol (Purdy et al, 1971; Ruder et al, 1972; Longcope et al, 1972). Because of significant diurnal variation in the secretion of androgens, the extent of the contribution to oestrogens by the peripheral conversion of androstenedione and testosterone measured under steady state conditions may have been overestimated (Baird et al, 1969a).

In this study, testicular oestrogen secretion was studied in men undergoing operative repair of varicocoeles by the simultaneous measurement of oestradiol, oestrone and oestrone sulphate concentrations in spermatic and peripheral veins.

4.2 Patients and methods

Seventeen patients (age 18-39, mean 26.2 $\pm$ 4.4 years) in good general health and who had received no regular medications prior to surgery underwent ligation of the left spermatic vein for treatment of varicocoeles. Eight of these patients presented with infertility while the other nine were treated for other symptoms associated with varicocoeles. Semen analyses were carried out only in those patients who presented with infertility. At least two semen samples from these patients were analysed and the average sperm density was used.

Blood was aspirated during the operation, which was performed under general anaesthesia by needle puncture simultaneously from the left spermatic vein (2-10 ml) and the antecubital vein (10-20 ml). The plasma was separated

by centrifugation and stored at -20°C until analysis. Blood samples were taken between 0900 and 1400 hours whilst the patients were in a horizontal position. Oestradiol, oestrone, oestrone sulphate, androstenedione and testosterone concentrations were measured in each spermatic vein and peripheral vein sample by the methods described in Appendix I.

Statistical analyses were performed using Student's one-tailed t-test and Kendall's rank correlation.

4.3 Results

The values of the five steroids in the spermatic and peripheral venous plasma and the gonadotrophins in peripheral venous plasma are shown in Table 4.1. The concentrations of testosterone, androstenedione, oestradiol and oestrone were significantly higher in the spermatic than peripheral veins (paired t-test $P<0.001$, $P<0.005$, $P<0.025$, $P<0.01$ respectively); the ratios of the mean steroid concentrations in spermatic to peripheral venous plasma were 77.2, 9.1, 28.7 and 1.6 respectively. Although the mean spermatic vein concentration of oestrone sulphate was significantly higher (paired t-test, $P<0.05$) than that in peripheral vein, the ratio of the mean spermatic and peripheral vein concentrations were not significantly different from unity. Furthermore, in five patients, the spermatic vein concentrations of oestrone sulphate were lower than those in the peripheral vein.

There were significant correlations (Kendall's rank

Patients	Age yrs	Sperm Density million/ml	Oestrone sulphate pg/ml		Oestrone pg/ml		Oestradiol pg/ml		Androstenedione ng/ml		Testosterone ng/ml		Gonadotrophins u/l	
			S	P	S	P	S	P	S	P	S	P	LH	FSH
1 GP	25		745	967	31	35	116	47	1.925	1.178	50.926	4.321	9.9	7.5
2 EM	22		1025	961	42	52	116	49	1.055	1.114	60.185	2.839	3.2	1.9
3 DD	18		1036	764	204	52	-	53	38.835	1.021	1913.58	4.444	5.6	2.6
4 AC	27	1.0	995	980	126	39	4516	36	38.674	1.283	864.198	3.877	10.8	16.3
5 BB	33	167.5	482	410	34	47	77	39	4.881	0.607	16.049	7.901	4.2	1.6
6 DA	27	1.5	759	743	51	48	3097	35	2.346	1.028	29.012	3.209	8.4	9.2
7 DM	23	26.0	1033	1042	119	44	90	29	5.525	1.670	132.716	2.840	2.2	1.0
8 PD	28		899	790	37	40	90	53	3.078	1.436	3.703	2.962	11.6	3.3
9 AD	24		1846	1882	83	68	77	71	1.701	0.813	17.283	4.444	10.1	5.9
10 WP	33		741	679	63	51	2000	42	14.077	1.654	648.148	3.950	14.8	7.1
11 AD	19		1349	1093	86	66	413	27	7.222	1.381	185.185	3.500	-	-
12 RB	30	122.0	922	821	112	68	2000	35	23.837	1.134	771.605	8.024	7.9	2.4
13 BW	27	7.3	685	573	98	58	5806	39	37.270	1.140	52.469	3.506	8.7	6.3
14 RF	23	20.2	1125	1197	99	51	645	56	11.764	1.687	203.704	4.938	3.5	5.2
15 HS	34	23.3	1268	942	37	50	116	36	1.580	3.984	3.086	6.172	4.7	3.5
16 JD	26		607	538	43	29	426	50	25.797	1.579	864.198	4.444	9.5	6.2
17 JC	27		1147	1201	75	30	-	73	-	2.921	197.531	6.666	9.0	5.3
(Mean ± SD)	26.2 (4.4)		980 (314)	917 (325)	78.8 (44)	48.7 (11.6)	1305 (1769)	45.3 (12.8)	13.722 (13.88)	1.508 (0.79)	353.74 (496.25)	4.59 (1.60)	7.76 (3.40)	5.2 (3.78)
Paired t test			p<0.05		p<0.01		p<0.025		p<0.005		p<0.001		-	
Spermatic: Peripheral ratio			1.07		1.61		28.8		9.10		77.1		-	

Table 4.1 Concentration of oestrone sulphate, oestrone, oestradiol, androstenedione and testosterone in spermatic (S) and antecubital (P) venous plasma and gonadotrophins in antecubital venous plasma with varicocoeles.

correlation $P<0.05$ - <0.01) between the testicular secretion as represented by the individual spermatic : peripheral venous concentrations in each of the 17 patients of oestrone, oestradiol, androstenedione and testosterone, but not of oestrone sulphate.

The blood production rate (P_B), defined as the total amount of steroid entering the circulation de novo, and testicular secretion rate (S) of the five steroids can be estimated from the known metabolic clearance rates (MCRs) in previous publications and the testicular blood flow (TBF) respectively. Based on the finding that testosterone enters the circulation entirely from testicular secretion (Hudson et al, 1967), it follows that blood production rate of this testicular steroid is the same as the testicular secretion rate. Consequently, the testicular blood flow (two testes combined) can be derived from the following formulations (Baird, 1971):

$$S = TBF \times (i_V - i_A) = P_B = MCR \times i_A$$

$$TBF = \frac{MCR \times i_A}{i_V - i_A}$$

$$= \frac{MCR}{r - 1}$$

where i_V is steroid concentration in spermatic vein, i_A is steroid concentration in spermatic artery or peripheral vein and r is the ratio of i_V/i_A. Applying this formula to the mean testosterone concentration in the spermatic and peripheral veins in the present study, the mean testicular blood flow was calculated to be 14.2 L/day or

Steroids	Metabolic Clearance Rate MCR L/day	Spermatic vein conc. i_V (pg/ml)	Peripheral vein conc. i_A (pg/ml)	Blood Production Rate P_B (μg/day)	Testicular Secretion Rate S (μg/day)
Oestrone sulphate	161[a]	980	917	147	0.9
Oestrone	2240[b]	79	49	109	0.4
Oestradiol	1760[b]	1305	45	80	18
Androstenedione	2300[c]	13722	1508	3468	173
Testosterone	1080[c]	353740	4590	4957	4957[d]

Table 4.2 The estimated blood production (P_B) and testicular secretion (S) rates of oestrone sulphate, oestrone, oestradiol, androstenedione and testosterone in men.

a : from Ruder et al (1972) and Longcope (1972)

b : from Longcope & Tait (1971)

c : from Baird et al (1968)

d : $S = P_B$ since virtually all testosterone in man originates from testicular secretion. The testicular blood flow is calculated to be 14.2 L/day or 9.86 ml/min (see text).

9.9 ml/min. This enabled the testicular secretion rates of the other steroids to be estimated from the spermatic and peripheral vein concentration differentials (Table 4.2).

The mean LH and FSH levels in the 17 patients with varicocoeles were within the appropriate normal ranges for adult males (LH 3.4-10 u/l and FSH 1.7-6.7 u/l). However, four patients (Nos. 1, 4, 6 & 10), one of whom was severely oligospermic, had raised plasma concentrations of FSH. The mean levels of various steroids in spermatic and peripheral venous blood in these four patients with elevated FSH were not significantly different from those with normal gonadotrophins although the mean spermatic vein concentration of oestradiol was higher in the former group (2432 ± 1607 vs 996 ± 1643 pg/ml).

4.4 Discussion

The mean peripheral venous concentration of oestrone sulphate in seventeen men with varicocoeles was 917 ± 325 (S.D.) pg/ml. This is comparable to values in previous studies in normal adult men, for example Hawkins and Oakey (1974): 716 ± 299 pg/ml; Wright et al (1978): 922 ± 62 pg/ml; Franz et al (1979): 458 ± 25 pg/ml and Towobola et al (1980): 1180 ± 225 pg/ml.

The demonstration of adrenal secretion of dehydroepiandrosterone sulphate (Baulieu et al, 1965) and the finding that sulphated steroids can interconvert without prior hydrolysis of the sulphate moiety (Lieberman, 1967)

prompted studies to investigate whether phenolic steroid conjugates are also secreted by steroid-producing glands or formed solely as a result of peripheral conversion of precursors. The present study demonstrated an insignificant testicular-peripheral vein concentration gradient for oestrone sulphate indicating, if spermatic artery concentration is equated with that in the peripheral vessels, that there is no testicular secretion of the conjugated oestrogen despite its relative abundance in the circulation. The blood production rate P_B (metabolic clearance rate x arterial concentration) of oestrone sulphate is estimated to be 147 µg/day (Table 4.2). This is two to three times higher than that reported by Ruder et al (1972) and Longcope (1972) whose calculations were based on the rather low mean peripheral plasma concentration of oestrone sulphate in the study of Loriaux et al (1971). The observation that the combined contributions of oestradiol and oestrone as precursors of oestrone sulphate (Ruder et al, 1972) considerably exceeded the calculated blood production rate of oestrone sulphate (Ruder et al, 1972; Longcope, 1972) suggested that these earlier estimates of blood production may be too low. Using the transfer constants (defined as the fraction of precursor which enters the blood de novo as the relevant product) from Ruder et al (1972) and Longcope (1972), it was possible to estimate, from the plasma concentrations in the present study, that the contribution of the two precursors to oestrone sulphate was 51 µg/day (oestradiol) and 73 µg/day (oestrone).

This indicates that virtually all of the plasma oestrone sulphate is derived from oestrone and oestradiol, confirming the insignificant contribution by testicular secretion. Since the error in such calculations is relatively high, it is possible that the adrenal gland is a further source of oestrone sulphate. Bovine adrenal tissue can synthesize oestrone sulphate in vitro (Sneddon & Marrian, 1963), but whether there is significant synthesis in vivo and secretion of oestrone sulphate by the human adrenal gland remains to be determined. Thus on present evidence, unlike dehydroepiandrosterone sulphate, oestrone sulphate is unlikely to be an important precursor for unconjugated oestrogens in either normal or infertile men (see later).

The blood production rate of oestrone is estimated to be 109 μg/day. This is in good agreement with previous studies (Baird et al, 1969a; Longcope, 1972; Ruder et al, 1972). The concentration of oestrone in the spermatic vein in this study is considerably lower than that reported by Baird et al (1973) and Weinstein et al (1974), but more comparable to that reported by Longcope et al (1972). In this latter study, unexpectedly low spermatic vein concentrations of androstenedione, testosterone and oestradiol were also found suggesting that the retrograde approach via the inferior vena cava and renal vein might have introduced a degree of dilution in the spermatic vein effluent. This is unlikely to be the case in the present study since the spermatic vein concentration of testosterone, androstenedione and

oestradiol were comparable to other reports (Laatikainen et al, 1971; Weinstein et al, 1974; Fiorelli et al, 1976; Hammond et al, 1977; Pirke et al, 1977; de la Torre et al, 1978). The correlation of spermatic-peripheral vein concentration ratios of the four unconjugated steroids further substantiates the validity of the present results. Thus the testicular secretion of oestrone in this study would appear to be smaller than previously found, with a contribution of under 1% to the daily blood production (Table 4.2). Since the contribution from androstenedione accounts for at most 50-60% of oestrone production rate, and the adrenal secretion is small - 10 μg/day (Baird et al, 1969b), a substantial proportion of oestrone blood production remains unaccounted for. The considerable variation in spermatic vein steroid concentration (coefficient of variation 33-134%) is not unexpected in view of the episodic nature of testicular hormone secretion (Smith et al, 1974) and the variability inherent in the anatomical level of operative approach to the spermatic vein, the duration and the negative pressure applied in blood sampling. Where the rate of testicular hormone secretion is low, as in oestrone, these variables may have disproportionate effects leading to underestimation. There does not seem to be any entirely satisfactory explanation for the discrepant results in testicular secretion of oestrone at present.

The peripheral vein concentration and hence the production rate of oestradiol in this study (Table 4.2) is higher than previous studies (Kelch et al, 1972; Baird

et al, 1973; Weinstein et al, 1974). However, the calculated testicular secretion of oestradiol amounted to 22% of blood production which is in good agreement with previous data (Kelch et al, 1972; Weinstein et al, 1974).

Both the peripheral plasma concentration and the calculated blood production rate of androstenedione are somewhat higher than values obtained in previous studies under basal conditions (Horton & Tait, 1966; Rivarola et al, 1966). In patients undergoing surgery this is not unexpected in view of the reports of increased plasma levels (Rivarola et al, 1966; Beitins et al, 1973; Irvine et al, 1974) and adrenal secretion (Baird et al, 1969b; Rosenfield, 1969) of androstenedione in response to ACTH stimulation or stress. The calculated testicular secretion is small (under 10% of 2 mg which is the generally accepted daily production rate under basal conditions). This contrasts with the female where ovarian secretion contributes 20-50% of the daily blood production of androstenedione (Baird, 1971; Baird et al, 1974). Peripheral conversion of testosterone and dehydroepiandrosterone is expected to produce no more than 0.9 mg of androstenedione daily (Horton & Tait, 1967). Thus it would appear that adrenal secretion provides the major share of androstenedione blood production in man. This conclusion is supported by earlier reports (Weinheimer et al, 1965; Wieland et al, 1965; Baird et al, 1969b; Rosenfield, 1969) confirming the substantial adrenal secretion of androstenedione with adrenal vein concentrations well in excess of that found

in the spermatic and peripheral veins.

The comparison of spermatic and peripheral venous concentrations of the five steroids between the four subjects with raised FSH and/or oligospermia and the rest of the study group with normal FSH and/or sperm densities showed only higher spermatic vein concentrations of oestradiol which just failed to reach the 5% level of statistical significance. This is compatible with the findings in Chapter 3 that men with severe disorders of spermatogenesis have increased levels of oestrogens in the peripheral circulation. The present findings would suggest that increased testicular secretion of oestradiol may be one of the mechanisms which contribute to this circulating oestrogen excess in men with elevated FSH secondary to seminiferous tubular damage. However, no difference in peripheral venous concentrations of the three oestrogens was demonstrated between men with normal or elevated FSH. This may be due to the small number of subjects studied, the relatively lower FSH levels in the subjects in this compared to the study in Chapter 3 and the rather small difference demonstrated in spermatic vein concentration of oestradiol. For these reasons, the present results must be regarded as preliminary and the association between elevated FSH and increased testicular oestrogen secretion tentative.

Although some reports claimed to have demonstrated abnormal Leydig cell function in patients with varicocoele, this has not been confirmed by others. Raboch and Starka (1971) and Comhaire & Vermeulen (1975) found lower

peripheral testosterone levels in patients with varicocoeles which correlated with increasing age. In vitro studies using testicular biopsy specimens showed reduced synthesis of ^{3}H-testosterone from ^{3}H-pregnenolone in 16 patients with varicocoeles compared with one normal control (Weiss et al, 1978). However, normal peripheral concentrations of gonadotrophins, testosterone and oestradiol were reported by Swerdloff & Walsh (1975), Schiff et al (1976) and Rege et al (1979). Baird et al (1973) and Swerdloff & Walsh (1975) also found comparable levels of testosterone and oestradiol in spermatic veins of patients with varicocoeles or inguinal hernias. The available evidence suggests that studies on testicular steroid secretion based on spermatic vein concentrations in patients with varicocoeles should only be extrapolated to the physiological state provided that the seminiferous tubular function has not been damaged to the extent that FSH becomes elevated. Furthermore, other factors which have not so far been taken into account are the suppressive effects of surgical stress (Eik-Nes, 1970; Wang et al, 1978) and anaesthesia (Carstensen et al, 1973) on Leydig cell function.

In summary, the present study showed minimal testicular secretion of oestrone sulphate, supporting the view derived from isotope dilution studies that almost all of this conjugated steroid is derived from peripheral conversion of other precursor steroids. There is preliminary evidence for increased testicular secretion of oestradiol in men with elevated FSH.

SECTION D

CONCLUSION AND OVERVIEW

This series of clinical studies in male patients with delayed puberty and infertility were conducted primarily to gain further insight into the pathophysiology of these two entities. The information obtained did improve the diagnostic efficiency and rationalized the clinical management in these patients. In the assessment of patients with delayed puberty, testicular volume was confirmed to be the most informative single clinical parameter which bore a constant relationship to the hormonal changes throughout puberty. Amongst the many hormonal measurements, the LH response to a single 10 μg bolus of exogenous GnRH provided the most representative information on the state of maturity of the HPT axis. Since pituitary responsiveness reflects previous exposure to GnRH, an immature response does not carry any predictive information. Thus hypogonadotrophic patients cannot be distinguished from those with constitutional delayed puberty on the strength of a single GnRH stimulation test. Basal concentrations of gonadotrophins including those at night and multiple GnRH stimulation did not yield any additional information clinically. In adult patients with defective spermatogenesis on the other hand, the GnRH response correlated so well with basal gonadotrophin concentrations that a single basal measurement of FSH, if it was elevated, was able to predict the presence of significant and probably irreversible germ cell loss/damage in the testis.

Routine measurement of LH, testosterone and oestrogen added little useful information in these normally virilized patients. These findings indicate that it is only when hormone deficiency is suspected that dynamic stimulatory tests may have advantages over the use of basal measurements; the latter usually suffices for the diagnosis of hormone excess.

Although the clinical information is helpful, it is the implications of these data to the normal homeostatic mechanisms in the hypothalamic-pituitary-testicular axis which generated most interest. Puberty should be regarded as the second half of a diphasic mechanism with the first half being the pre and neonatal sexual differentiation. Interposed between the two is a long period of physical and mental growth with quiescent sexual function. The reawakening of the sexual developmental process may be a function of the maturity of the whole body and in particular the brain. Although the ontogenic function of sexual differentiation and development is genetically programmed, the timing and the individual components in the system are influenced by epigenetic and environmental factors. The triggering mechanism and its timing for the initiation of the second phase of sexual development is still unclear. This study confirmed that the initiation of spontaneous puberty is intimately associated with neuronal mechanisms (probably via neurotransmitters and/or neuronal peptides) that also control sleep. Thus, the prepubertal neuroinhibitory mechanisms acting on the hypothalamic

"pulse generator" become suppressed during sleep in early puberty, allowing the uninhibited discharge of GnRH-secreting neurones in the arcuate nucleus. Accordingly, the primary neuronal signal in the afferent neurones supplying the arcuate nucleus is frequency-coded and is translated into an endocrine signal by the GnRH-secreting neurones which discharge intermittently into the privileged channels of the hypophysial portal circulation. The pituitary gonadotropes are sensitive to the frequency changes of the pulsative GnRH stimulation but the alterations in amplitude in the hypophysial portal circulation seem of less consequence. Thus the change in frequency of neuroendocrine discharge of the hypothalamus is the principal mechanism by which the neuronal reproductive signal of the brain is translated into a hormonal message of the HPT axis. At the pituitary, the neuroendocrine signal is amplified a few thousand-fold in order to preserve its temporal pattern in the systemic circulation. Optimal function of the gonads during puberty may be dependent both on the amplitude and frequency of LH pulsatile stimulation. Testosterone levels in the peripheral circulation of man do not show distinct episodic fluctuations which are temporally synchronized to that of LH. Although the explanation for this is unclear, it does imply that physiological functions in the androgen-responsive target cells may be independent of the pulsatile signal pattern which is critical for pituitary regulation. Thus as the "reproductive message" is transmitted from the higher

Site	Hormonal Messenger	Pattern of Signalling
Brain		
	Neurotransmitter amine / peptide	Discharge frequency
Hypothalamus		
	GnRH	Pulse frequency
Pituitary		
	LH	Pulse amplitude & frequency
Testis		
	Testosterone	"non-pulsatile"
Target cells		

Table 1

The pattern of hormonal signalling in the Brain-Hypothalamus-Pituitary-Testicular axis. The frequency mode gives way to amplitude mode as the hormone signal is transmitted down towards the target cells which are exposed to testosterone levels which show no distinct pulsatile pattern.

centres down the hypothalamic-pituitary-testicular axis to the target cells, there is a gradual transformation of the pattern of signals from frequency to amplitude mode (Table 1). By the time the hormonal signal reaches the target cells, these androgen-sensitive tissues are exposed to more or less constant levels of sex steroids in which the pulse pattern generated by the arcuate nucleus is no longer detectable.

The disruption of normal spermatogenesis in adult patients with infertility also provided information compatible with the existence of intratesticular regulatory mechanisms which so far has only been demonstrated in experimental animals. Although the information is mainly based on circulating hormone concentrations, spermatic vein sampling brought us one step nearer towards the study of control mechanisms within secretory and target organs in the HPT axis. The successful completion of spermatogenesis is dependent on the triangular relationship between germ cells, Sertoli cells and Leydig cells in the testis. Mediating their interactions may be both stimulatory as well as inhibitory factors including testosterone, oestradiol, GnRH-like testicular peptide and Sertoli cell factor - all functioning as local intratesticular hormones. On present evidence, it is probable that both circulating or local hormones exert their effects on germ cells via the Sertoli cells. Thus it is conceivable that the interaction between Leydig and Sertoli cells, via the local regulatory factors, can ensure a stable biochemical

milieu in the adluminal compartment of the seminiferous tubules where meiosis of the spermatocytes can be completed and the various steps of spermiogenesis can take place. In this way, the primary reproductive signal is decoded at the hypothalamus and the effects of the fluctuating stimulus is smoothed out by the intratesticular regulatory mechanisms and perhaps androgen binding protein in order to effect the continuous production of mature spermatozoa. An analagous situation where the stable action of steroid is more effective than an intermittent one is seen in the negative feedback of LH secretion and stimulation of accessory glands by testosterone (Damassa et al, 1976; Smith et al, 1977). The existence of sex hormone binding globulin may also contribute in this respect to the action of testosterone on other target organs.

By virtue of the proximity of the seminiferous tubules to the Leydig cells, the former is the only androgen target tissue which can directly feed back on their source of supply of steroid hormone and modify its function. This highlights the crucial role of androgen in spermatogenesis and further emphasizes the physiological significance of intratesticular regulatory mechanisms. Since the first wave of spermatogenesis is established very soon after the formation of spermatogonia with appearance of spermatozoa in the lumen of seminiferous tubules in most species studied, it follows that the intratesticular regulatory mechanisms must be established together with feedback mechanisms at the pituitary at a

very early stage during pubertal development. The finding that FSH secretion in the pituitary becomes suppressed with the first signs of testicular enlargement is compatible with this view. Once the effects of FSH on Sertoli and Leydig cell maturation is accomplished in early puberty, the imminent suppression of FSH and the relative insensitivity of adult Sertoli cells to FSH stimulation can be regarded as a double safeguard against excessive FSH-induced oestrogen production which may be deleterious to both Leydig and germ cells (Libbus & Scheutz, 1979 & 1980). These safety mechanisms may be overcome under pathological situations as shown here by the increased circulating levels of oestrogens in men with elevated FSH. Any disturbance of the delicately-balanced interrelationship between Sertoli and Leydig cells during the establishment of spermatogenesis in the peripubertal period may ultimately lead to defective spermatogenesis in adult life which may not be associated with any structural or circulatory hormonal abnormalities.

In the last decade, the traditional boundaries of endocrinology have been greatly expanded. The concept that a hormone is the unique product of a specific cell type in an endocrine gland is no longer tenable. The discovery of hormone secreting neurones and a wide variety of hormonal peptides in the brain and gut tissues as well as unicellular organisms has led to a complete reshaping of the current concepts of the endocrine system (Roth et al, 1982; Kruger & Martin, 1981). The new-found knowledge regarding the functions of the brain-

hypothalamus-pituitary-gonadal axis has provided prominent examples towards the redefining of biochemical messengers used in vertebrates as mechanisms of intercellular communication. Thus the functions of the classical endocrine glands of pituitary and testes are seen to overlap with those of the central nervous system, paracrine agents and exocrine secretions. The GnRH secretory neurones in the hypothalamus under the controlled neurotransmitter amines play a key role in the neuroendocrine control of reproductive function. This is probably the best example to date that the central nervous system not only functions as part of the endocrine system but is also the means for adapting diverse body functions constantly to changes in the environment. The brain itself is a target organ for gonadal steroids in the organization and activation of sexual behaviour and in the negative feedback control of GnRH secretion. Paracrine agents or local tissue factors in the form of GnRH-like peptides, oestradiol, Sertoli cell factor, Mullerian-Inhibiting-Factor show the diversity of intratesticular control mechanisms. Although we are just beginning to identify the mechanisms of control and action of these paracrine agents, there is little doubt that they are important in the physiological regulation of normal testicular function. Lastly, the testis has long been recognized as an organ which combines endocrine and exocrine functions. What was not known until recently is the intimate interrelationship between the Leydig cells and seminiferous tubular function. In the

Sertoli cells reside the principal source of a wide variety of exocrine secretory products which may prove to execute the hormonal control of spermatogenesis. Viewed in this new light, the endocrine system can be regarded as a system made up of intercellular biochemical messengers relaying metabolic signals and commands between secretory and target cells. In the case of the male reproductive function, the biological process is made up of a hierarchical system of biochemical communication and control between the brain and the genome in target cells. In between these lies a series of stages of information transfer including (1) genome-ribosome communications, (2) job control at genome level, (3) metabolite flow, (4) second messenger and protein phosphorylation, (5) steroid signals, (6) amino acid derivative signals, (7) polypeptide signals, (8) glycoprotein/glycolipid receptors-receptor interaction at cell surfaces, (9) gap junction network convections and (10) neural net electrochemical pulsing (Yates, 1981). It is clear that many forms of co-operation at multiple levels are required to support this life function. The study of hormonal signal dynamics in blood is only one aspect of the entire system of intercellular communication. There are obviously many other equally important facets of male reproductive function or dysfunction which await the same endeavour we have paid to urinary or circulating hormone concentrations.

REFERENCES

1 Aafjes JH, van der Vijver JCM (1974) A relationship between testicular biopsy score count and fertility of men treated for oligospermia. Fertil Steril 25: 809-812.

2 Aafjes JH, van der Vijver JCM, Docter R, Schenk PE (1977) Serum gonadotrophins, testosterone and spermatogenesis in subfertile men. Acta Endocrinol 86:651-658.

3 Aafjes JH, van der Vijver JCM, Schenk PE (1978) Value of a testicular biopsy rating for prognosis in oligospermia. Br Med J 1:289-290.

4 Abraham GE (1969) Solid-phase radioimmunoassay of oestradiol-17β. J Clin Endocrinol Metab 29:866-870.

5 Adam K, Oswald I (1977) Sleep is for tissue restoration. J R Coll Physicians Lond 11:376-388.

6 Ahmad N, Italtemeyer C, Eik-Nes KB (1973) Maintenance of spermatogenesis in rats with intratesticular implants of testosterone or dihydrotestosterone. Biol Reprod 8:411-419.

7 Aiman J, Griffen JE, Gonak YM, Wilson JD, MacDonald PC (1979) Androgen insensitivity as a cause of male infertility in otherwise normal men. N Engl J Med 300:223-227.

8 Aiman J, Hemsell DL, MacDonald PC (1978) Production and origin of estrogen in two true hermaphrodites. Am J Obstet Gynecol 132:401-409.

9 Aiyer MS, Chiappa SA, Fink G (1974) A priming effect of LH-releasing factor on the anterior pituitary in the female rat. J Endocrinol 62:573-588.

10 Albert A, Underdahl LO, Greene LF, Lorenz N (1953) Male hypogonadism I. The normal testis. Proc Staff Meetings Mayo Clinic 28:409-422.

11 Alford FP, Baker HWG, Burger HG, de Kretser DM, Hudson B, Johns MW, Masterton JP, Patel YC, Rennie GC (1973) Temporal patterns of integrated plasma hormone levels during sleep and wakefulness II. Follicle-stimulating hormone, luteinizing hormone, testosterone and oestradiol. J Clin Endocrinol Metab 37:848-854.

12 Aloysio D, Busacchi P, Bolelli GF, Vechi F, Flamigni C (1974) Radioimmunological assay of testosterone and gonadotrophins in plasma and seminal fluid of healthy subjects and patients with dysspermia. Acta Eur Fertil 5:317-351.

13 Amelar RD, Hotchkiss RS (1963) Congenital aplasia of the epididymides and vasa deferentia: effects on semen. Fertil Steril 14:44-48.

14 Anderson DC, Lasley BL, Fisher BA, Shepperd JH, Newman L, Hendrickx AG (1976) Transplacental gradients of sex-hormone-binding-globulin in human and simian pregnancy. Clin Endocrinol 5:657-669.

15 Anderson KM, Liao S (1968) Selective retention of dihydrotestosterone by prostatic nuclei. Nature 219: 277-279.

16 Angsusingha K, Kenny F, Nankin HR (1974) Unconjugated estrone, estradiol, and FSH and LH in prepubertal and pubertal males and females. J Clin Endocrinol Metab 39:63-68.

17 Anton-Tay F, Diaz JL, Fernandez-Guardida A (1971) On the effect of melatonin upon human brain. Its possible therapeutic implications. Life Sci 10: 841-850.

18 Aragona C, Bohnet HG, Friesen HG (1977) Localization of prolactin binding in prostate and testis: the role of serum prolactin concentration on the testicular LH receptor. Acta Endocrinol 84:402-409.

19 Arai Y, Matsumoto A (1978) Synapse formation of the hypothalamic arcuate nucleus during post-natal development in the female rat and its modification by neonatal estrogen treatment. Psychoneuroendocrinology 3:31-45.

20 Araki S, Toran-Allerand CD, Ferin M, Vande Wiele RL (1975) Immunoreactive gonadotrophin-releasing hormone (GnRH) during maturation in the rat: ontogeny of regional hypothalamic differences. Endocrinology 97:693-697.

21 Arendash GW, Gallo RV (1978) Apomorphine-induced inhibition of episodic LH release in ovariectomized rats with complete hypothalamic differentiation. Proc Soc Exp Biol Med 159:121-125.

22 Arimura A (1977) Hypothalamic gonadotrophin-releasing hormone and reproduction. In: Reproductive Physiology II. Ed: RO Greep. University Park Press, Baltimore. Int Rev Physiol 13, ppl-21.

23 Arimura A, Kastin AJ, Gonzalez-Barcena D, Siler J, Weaver RE, Schally AV (1974) Disappearance of LH-releasing hormone in man as determined by radio-immunoassay. Clin Endocrinol 3:421-425.

24 Armstrong DT, Dorrington JH (1977) Estrogen biosynthesis in the ovaries and testes. Adv Sex Horm Res 3:217-258.

25 Arslan M, Zaidii AS, Quazi MH (1978) Effect of gonadotrophins and LH-RH on functional differentiation of immature monkey testis. Int J Androl (Suppl) 2:319-328.

26 Ascoli M, Puett D (1976) Biotransformation of pituitary luteinizing hormone in serum and urine II. Evidence for reduced potency following urinary extraction. Endocrinology 99:1237-1243.

27 Attramadal A, Weddington SC, Naess O, Djøseland O, Hansson V (1976) Androgen binding and metabolism in the human prostate. In: Prostatic Disease. Eds: H Marberger, H Haschek, HKA Schirmer, JAC Colston, E Witkin. Alan Liss, New York, pp189-203.

28 Aubert ML, Sizonenko PC, Kaplan SL, Grumbach MM (1977) The ontogenesis of human prolactin from fetal life to puberty. In: Prolactin and Human Reproduction Eds: PG Crosignani, C Robyn. Academic Press, New York, pp9-20.

29 August GP, Grumbach MM, Kaplan SL (1972) Hormonal changes in puberty III. Correlation of plasma testosterone LH, FSH testicular size and bone age with male pubertal development. J Clin Endocrinol Metab 34:319-326.

30 August GP, Tkachuk M, Grumbach MM (1969) Plasma testosterone-binding affinity and testosterone in umbilical cord plasma, late pregnancy, prepubertal children and adults. J Clin Endocrinol Metab 29: 891-899.

31 Axelrod LR (1965) Metabolic patterns of steroid biosynthesis in young and aged human testes. Biochim Biophys Acta 97:551-556.

32 Bagshawe KD (1975) Computer controlled automated radioimmunoassay. Laboratory Practice, September: 573-575.

33 Baird DT (1971) Steroids in blood reflecting ovarian function. In: Control of Gonadal Steroid Secretion. Eds: DT Baird, JA Strong. Edinburgh University Press, Edinburgh, pp176-189.

34 Baird DT, Burger PE, Heavon-Jones GD, Scaramuzzi RJ (1974) The site of secretion of androstenedione in non-pregnant women. J Endocrinol 63:201-212.

35 Baird DT, Galbraith A, Fraser I, Newsam JE (1973) The concentration of oestrone and oestradiol-17β in spermatic veinous blood in man. J Endocrinol 57: 285-288.

36 Baird DT, Horton R, Longcope C, Tait JF (1968) Steroid prehormones. Perspect Biol Med 11:384-421.

37 Baird DT, Horton R, Longcope C, Tait JF (1969a) Steroid dynamics under steady-state conditions. Recent Prog Horm Res 25:611-664.

38 Baird DT, Uno A, Melby JC (1969b) Adrenal secretion of androgens and oestrogens. J Endocrinol 45:135-136.

39 Baker HWG, Bremner WJ, Burger HG, de Kretser DM, Dulmanis A, Eddie LW, Hudson B, Keogh EJ, Lee VWK, Rennie GC (1976) Testicular control of follicle-stimulating hormone secretion. Recent Prog Horm Res 32:429-477.

40 Baker HWG, Burger HG, de Kretser DM, Dulmanis A, Hudson B, O'Connor S, Paulsen CA, Purcell N, Rennie GC, Seah CS, Taft HP, Wang C (1976) A study of the endocrine manifestations of hepatic cirrhosis. Q J Med 45:145-178.

41 Baker HWG, Burger HG, de Kretser DM, Hudson B, O'Connor S, Wang C, Mirovics A, Court J, Dunlop M, Rennie GC (1976) Changes in the pituitary testicular system with age. Clin Endocrinol 5:349-372.

42 Baker HWG, Santen RJ, Burger HG, de Kretser DM, Hudson B, Pepperell RJ, Bardin CW (1975) Rhythms in the secretion of gonadotrophins and gonadal steroids. J Steroid Biochem 6:793-801.

43 Bambino TH, Schreiber JR, Hsueh AJW (1980) Gonadotrophin-releasing hormone and its agonist inhibit testicular luteinizing hormone receptor and steroidogenesis in immature and adult hypophysectomized rats. Endocrinology 107:908-917.

44 Barbarino A, De Marinis L (1980) Estrogen induction of luteinizing hormone release in castrated adult human males. J Clin Endocrinol Metab 51:280-286.

45 Barbarino A, De Marinis L, Lafuenti G, Muscatello P, Matteucci BR (1979) Presence of positive feedback between oestrogen and LH in patients with Klinefelter's syndrome and Sertoli-cell-only syndrome. Clin Endocrinol 10:235-242.

46 Bardin CW, Catterall JF (1981) Testosterone: a major determinant of extragenital sexual dimorphism. Science 211:1285-1294.

47 Bardin CW, Mahoudeau JA (1970) Dynamics of androgen metabolism in women with hirsutism. Ann Clin Res 2: 251-262.

48 Barry J, Cotte G (1961) Etude préliminaire au microscope éléctronique de l'éminence mediane cobaye. Z Zellforsch mikrosk Anat 53:714-724.

49 Barry M (1843) Spermatozoa observed within the mammaliferous ovum. Embryological memoirs. Philos Trans R Soc Lond 128.

50 Bartke A (1969) Prolactin changes colesterol stores in the mouse testis. Nature 224:700-701.

51 Bartke A (1971a) Effects of prolactin on spermatogenesis in hypophysectomized mice. J Endocrinol 49: 311-316.

52 Bartke A (1971b) Effects of prolactin and luteinizing hormone on the cholesterol stores in the mouse testis. J Endocrinol 49:317-324.

53 Bartke A (1976) Pituitary-testis relationships role of prolactin in the regulation of testicular function. Prog Reprod Biol 1:136-150.

54 Bartke A, Hafiez AA, Bex FJ, Dalterior S (1978) Hormonal interactions in regulation of androgen secretion. Biol Reprod 18:44-54.

55 Baulieu EE, Corpechot C, Dray F, Emiliozzi R, Lebeau M-C, Mauvais-Jarvis P, Robel P (1965) An adrenal-secreted "androgen": dehydroepiandrosterone sulphate. Its metabolism and a tentative generalization on the metabolism of other steroid conjugates. Recent Prog Horm Res 21:411-500.

56 Beale EG, Dedman JR, Means AR (1977) Isolation and characterization of a protein from rat testis which inhibits cyclic AMP dependent protein kinase and phosphodiesterase. J Biol Chem 252:6322-6327.

57 Beall D (1940) The isolation of α oestradiol and oestrone from horse testes. Biochem J 34:1293-1298.

58 Beck K (1971) Morphology of the fetal adrenal cortex and maternal urinary oestriol excretion in pregnancy. Acta Obstet Gynecol Scand 50:215-222.

59 Beck W, Wuttke W (1980) Diurnal variation of plasma luteinizing hormone, follicle-stimulating hormone, and prolactin in boys and girls from birth to puberty. J Clin Endocrinol Metab 50:635-639.

60 Behrman SJ, Kistner RW (1975) A rational approach to the evaluation of infertility. In: Progress in Infertility, 2nd edition. Eds: SJ Behrman, RW Kistner Little Brown & Co, Boston.

61 Beitins IZ, Bayard F, Kowarski A, Migeon CJ (1973) The effect of ACTH administration on plasma testosterone, dihydrotestosterone and serum LH concentrations in normal men. Steroids 21:553-564.

62 Beitins IZ, O'Loughlin K, Ostrea T, McArthur JW (1976) Gonadotropin determination in timed 3-hour urine collections during menstrual cycle and LHRH-testing. J Clin Endocrinol Metab 43:46-55.

63 Belchetz PE, Plant TM, Nakai Y, Keogh EG, Knobil E (1978) Hypophysial responses to continuous and intermittent delivery of hypothalamic GnRH. Science 202: 631-633.

64 Bell JBG, Lacy D (1974) Studies on the structure and function of the mammalian testes V. Steroid metabolism by isolated interstitium and seminiferous tubules of the human testes. Proc R Soc Lond (Biol) 186:99-120.

65 Bell J, Spitz I, Slonim A, Perlman A, Segal S, Palti Z, Rabinowitz D (1973) Heterogeneity of gonadotrophin response to LH-RH in hypogonadotropic hypogonadism. J Clin Endocrinol Metab 36:791-794.

66 Ben-Jonathan N, Oliver C, Weiner HJ, Mical RS, Porter JC (1977) Dopamine in hypophysial portal plasma of the rat during the estrous cycle and throughout pregnancy. Endocrinology 100:452-458.

67 Benson B (1977) Current status of pineal peptides. Neuroendocrinology 24:241-258.

68 Berg AA, Kjessler B, Liendkriest K (1976) In vitro metabolism of ^{3}H-progesterone in human testicular tissue. Acta Endocrinol (Suppl) 207:23-35.

69 Bergquist C, Nilius SG, Bergh T, Skarin G, Wide L (1979) Inhibitory effect on gonadotrophin secretion and gonadal function in men during chronic treatment with a potent stimulatory luteinizing hormone-releasing hormone analogue. Acta Endocrinol 91: 601-608.

70 Berlanger A, Cusan L, Auclair C, Seguin C, Caron S, Labrie F (1980) Effect of an LHRH agonist and hCG on testicular steroidogenesis in the adult rat. Biol Reprod 22:1094-1101.

71 Bermudez JA, Strott CA, Lipsett MB (1969) Blood levels and production rates of 17 hydroxypregnenolone. Clin Res 17:587.

72 Berson SA, Yalow RS (1970) Plasma insulin. In: Diabetes Mellitus: Theory and Practice. Eds: M Ellenberg, H Rifkin. McGraw-Hill, New York, pp308-367.

73 Berson SA, Yalow RS, Bauman A, Rothschild MA, Newerly K (1956) Insulin-^{131}I metabolism in human subjects: demonstration of insulin binding globulin in circulation of insulin treated subjects. J Clin Invest 35:170-190.

74 Berthold AA (1849) Transplantation der Hoden. Arch Anat Physiol Wiss Med 16:42-44.

75 Beyer C, Larsson K, Perez-Palacios G, Morali G (1973) Androgen structure and male sexual behaviour in the castrated rat. Horm Behav 4:99-108.

76 Bhattacharya AN, Dierschke DJ, Yamaji T, Knobil E (1972) The pharmacologic blockade of the circhoral mode of LH secretion in the ovariectomized rhesus monkey. Endocrinology 90:778-786.

77 Bidlingmaier F, Wagner Bannack M, Butenandt O, Knorr D (1973) Plasma oestrogens in childhood and puberty under physiologic and pathologic conditions. Pediatr Res 7:901-907.

78 Billewicz WZ, Fellowes HM, Thomson AM (1980) Post-menarchal menstrual cycle in British (Newcastle upon Tyne) girls. Ann Hum Biol 7:177-180.

79 Bird CE, Choong A, Knight L, Clark AF (1974) Kinetics of 5α-androstane-3α,17β-diol metabolism in normal men and women. J Clin Endocrinol Metab 38: 372-375.

80 Bird CE, Morrow L, Fukumoto L, Marcellus S, Clark AF (1976) Δ^5 Androstanediol: Kinetics of metabolism and binding to plasma proteins in normal men and women. J Clin Endocrinol Metab 43:1317-1322.

81 Blanc MR, Cahoreau C, Courot M, Dacheux JL, Hochereau-de-Revier MT, Pisselet C (1978) Plasma follicle stimulating hormone (FSH) and luteinizing hormone (LH) suppression in the cryptorchid ram by a non-steroidal factor (inhibin) from ram rete testis fluid. Int J Androl (Suppl) 2:139-146.

82 Blizzard RM, Penny R, Foley Jr TP, Baghdassarian A, Johnson A, Yen SSC (1972) Pituitary gonadal interrelationships in relation to puberty. In: Gonadotropins. Eds: BB Saxena, CG Beling, HM Gandy. John Wiley & Son, New York, pp502-523.

83 Bouin P, Ancel P (1903) Recherches sur les cellules interstitielle du testicule des mammiferes. Arch Zoo Exp Gen 1:437-523.

84 Boyar RM (1978) Control of the onset of puberty. Annu Rev Med 29:509-520.

85 Boyar R, Finkelstein J, Roffwarg H, Kapen S, Weitzman E, Hellman L (1972b) Synchronization of augmented LH secretion with sleep during puberty. N Engl J Med 287:582-586.

86 Boyar RM, Finkelstein JW, Roffwarg H, Kapen S, Weitzman ED, Hellman L (1973) Twenty-four hour luteinizing hormone secretory patterns in gonadal dysgenesis. J Clin Endocrinol Metab 37:521-525.

87 Boyar RM, Katz J, Finkelstein JW, Kapen S, Weiner ED, Hellman L (1974b) Anorexia nervosa: immaturity of the 24 hour luteinizing hormone secretory pattern. N Engl J Med 291:861-865.

88 Boyar R, Perlow M, Hellman L, Kapen S, Weitzman ED (1972a) Twenty-four hour pattern of luteinizing hormone secretion in normal men with sleep stage recording. J Clin Endocrinol Metab 35:73-81.

89 Boyar RM, Rosenfeld RD, Kapen S, Finkelstein J, Roffwarg HP, Weitzman ED, Hellman L (1974a) Human puberty: simultaneous augmented secretion of luteinizing hormone and testosterone during sleep. J Clin Invest 54:609-618.

90 Boyar RM, Wu RHK, Kapen S, Hellman L, Weitzman ED (1976) Clinical and laboratory heterogeneity in idiopathic hypogonadotrophic hypogonadism. J Clin Endocrinol Metab 43:1268-1274.

91 Bramble FJ, Broughton AC, Whittam TR, Eccles SS, Murray MAF, Jacobs HS (1975) Independent control of follicle-stimulating hormone and luteinizing hormone in man: results of unilateral orchidectomy. J Endocrinol 65:11P.

92 Bremner WJ, Findlay JK, Cumming IA, Hudson B, de Kretser DM (1976) Pituitary-testicular responses in rams to prolonged infusions of luteinizing hormone-releasing hormone (LHRH). Biol Reprod 15:141-146.

93 Bremner WJ, Paulsen CA (1974) Two pools of luteinizing hormone in human pituitary: evidence from constant administration of luteinizing hormone-releasing hormone J Clin Endocrinol Metab 39:811-815.

94 Brinkman AO, Leemborg FG, Roodnat EM, de Jong FH, van der Molen HJ (1980) A specific action of estradiol on enzymes involved in testicular steroidogenesis. Biol Reprod 23:801-809.

95 Brinkman AO, Mulder E, Lamers-Stahlhofen GJM (1972) An oestradiol receptor in rat testis interstitial tissue. FEBS Lett 26:301-305.

96 Brook CGD (1981) Delayed puberty. Br J Hosp Med 26: 573-580.

97 Brown GH, Seeman P, Lee T (1976) Dopamine/neuroleptic receptors in basal hypothalamus and pituitary. Endocrinology 99:1407-1410.

98 Brown JB, Smythe BJ (1971) Oestrone sulphate - the major circulating oestrogen in the normal menstrual cycle? J Reprod Fertil 24:142.

99 Brown PS (1958) Human urinary gonadotrophins I. In relation to puberty. J Endocrinol 17:329-336.

100 Brown-Grant K (1977) Physiological aspects of the steroid hormone-gonadotropin interrelationship. Int Rev Physiol, Reproductive Physiology II, Vol 13. Ed: RO Greep. University Park Press, Baltimore, pp57-83.

101 Brown-Sequard CE (1889) Des effets produits chez l'homme par des injections souscutanées d'un liquide retiré des testicules frais de cobaye et de chien. C R Seances Soc Biol 1:420-430.

102 Brownstein M (1977) Neurotransmitters and hypothalamic hormones in the central nervous system. Fed Proc 36: 1960-1963.

103 Bruchovsky N, Wilson JD (1968) The conversion of testosterone to 5α-androstan-17β-ol-3-one by rat prostate in vivo and in vitro. J Biol Chem 243: 2012-2021.

104 Burger HG, Baker HWG, de Kretser DM, Hudson B, Franchimont P, Pepperell RJ (1972) Regulation of gonadal functions in the male by gonadotropins. In: Gonadotropins and Gonadal Function. Ed: WR Moudgal. Academic Press, New York, pp531-544.

105 Burgus R, Butcher M, Amoss M, Ling N, Monahan M, Rivier J, Fellows R, Blackwell R, Vale W, Guillemin R (1972) Primary structure of the ovine hypothalamic luteinizing-hormone-releasing factor (LRF). Proc Nat Acad Sci USA 69:278-282.

106 Burr IM, Sizonenko PC, Kaplan SL, Grumbach MM (1970) Hormonal changes in puberty I. Correlation of serum luteinizing hormone and follicle stimulating hormone with stages of puberty, testicular size and bone age in normal boys. Pediatr Res 4:25-35.

107 Burton RM, Westphal U (1972) Steroid-hormone binding proteins in blood plasma. Metabolism 21:253-276.

108 Butenandt A (1931) Uber die chemische untersuchungen der Sexualhormone. Z F Angen Chem 44:905-908.

109 Butenandt A, Hanisch G (1935) Uber Testosteron. Unwandlung des Dehydroandrosterons in Androstenediol und Testosteron; ein Weg zur Darstellung des Testosteron aus Cholesterin. Z Physiol Chem 237: 89-97.

110 Buxton CL, Southam AL (1958) Human Infertility. Cassell, London, pp7, 60.

111 Byrnes WW, Meyer RK (1951) Effect of physiological amounts of estrogen on the secretion of follicle-stimulating and luteinizing hormones. Endocrinology 49:449-460.

112 Caligaris L, Astrada JJ, Taleisnik S (1972) Influence of age on the release of luteinizing hormone induced hy oestrogen and progesterone in immature rats. J Endocrinol 55:97-103.

113 Caminos-Torres R, Ma L, Snyder PJ (1977) Testosterone-induced inhibition of the LH and FSH responses to gonadotropin-releasing hormone occurs slowly. J Clin Endocrinol Metab 44:1142-1153.

114 Campbell HJ, Fever G, Garcia J, Harris GW (1961) The infusion of brain extracts into the anterior pituitary gland and the secretion of gonadotropic hormone. J Physiol 157:30P-31P.

115 Canick JA, Makris A, Gunsalus GL, Ryan KJ (1979) Testicular aromatization in immature rats: localization and stimulation after gonadotropin administration in vivo. Endocrinology 104:285-288.

116 Carmel PW, Araki S, Ferin M (1976) Pituitary stalk portal blood collection in Rhesus monkeys: evidence for pulsatile release of gonadotrophin releasing hormone (GnRH). Endocrinology 99:243-248.

117 Carstensen H, Amer I, Wide L, Amer B (1973) Plasma testosterone, LH and FSH during the first 24 h after surgical operations. J Steroid Biochem 4:605-611.

118 Carter JN, Tyson JE, Tolis G, Van Vliet S, Faiman C, Friesen HG (1978) Prolactin-secreting tumour and hypogonadism in 22 men. N Engl J Med 299:847-852.

119 Catt KJ, Dufau ML (1973) Spare gonadotrophin receptors in rat testis. Nature (New Biol) 244: 219-221.

120 Catt KJ, Dufau ML (1976) Basic concepts of the mechanism of action of peptide hormones. Biol Reprod 14:1-15.

121 Catt KJ, Harwood JP, Aguilera G, Dufau ML (1979) Hormonal regulation of peptide receptors and target cell responses. Nature 280:109-116.

122 Catt KJ, Dufau ML, Tsuruhara T (1972b) Radioligand-receptor assay of luteinizing hormone and chorionic gonadotrophin. J Clin Endocrinol Metab 34:123-132.

123 Catt KJ, Harwood JP, Clayton RN, Davies TF, Chan V, Katikineni M, Nozu K, Dufau ML (1980) Regulation of peptide hormone receptors and gonadal steroidogenesis. Recent Prog Horm Res 36:557-621.

124 Catt KJ, Tsuruhara T, Dufau ML (1972a) Gonadotrophin binding sites of the rat testis. Biochim Biophys Acta 279:194-201.

125 Catt KJ, Tsuruhara T, Mendelson C, Ketelslegers JM, Dufau ML (1974) Gonadotrophin binding and activation of the interstitial cells of the testis. In: Current Topics in Molecular Endocrinology I. Hormone Binding and Target Cell Activation in the Testis. Eds: ML Dufau, AR Means. Plenum Press, New York, ppl-30.

126 Cattanach BM, Pollard CE, Hawkes SG (1971) Sex reversed mice: XX and XO males. Cytogenetics 10: 318-337.

127 Chan L, O'Malley BW (1976) Mechanisms of action of the sex steroid hormones. N Engl J Med 294:1322-1328, 1372-1381, 1430-1437.

128 Chandley AC, Maclean N, Edmond P, Fletcher J, Watson GS (1976) Cytogenetics and infertility in man II. Testicular histology and meiosis. Ann Hum Genet 40: 165-176.

129 Chappel SC, Barraclough CA (1976) Hypothalamic regulation of pituitary FSH secretion. Endocrinology 98:927-935.

130 Chappel SC, Barraclough CA (1977) Further studies on the regulation of FSH secretion. Endocrinology 101: 24-31.

131 Charny CW (1940) Testicular biopsy; its value in male sterility. JAMA 115:1429-1432.

132 Charny CW, Conston AS, Meranze DR (1952) Testicular developmental histology. Ann NY Acad Sci 55:597-608.

133 Charreau EH, Attramadal A, Torjesen PA, Purvis K, Calendra R, Hansson V (1977) Prolactin bindin in rat testis: specific receptors in interstitial cells. Mol Cell Endocrinol 6:303-307.

134 Chasalow F, Marr H, Haour F, Saez JM (1979) Testicular steroidogenesis after human chorionic gonadotropin desensitization in rats. J Biol Chem 254:5613-5617.

135 Chaussain JL, Garnier PE, Binet E, Vassal J, Schöller R, Job JC (1974) Effect of synthetic LHRH on the release of gonadotrophins in hypophysogonadal disorders of children and adolescents III. Hypopituitarism. J Clin Endocrinol Metab 38:58-64.

136 Chemes HE, Dym M, Raj HGM (1979) The role of gonadotrophins and testosterone on initiation of spermatogenesis in the immature rat. Biol Reprod 21:241-249.

137 Chemes HE Rivarola MA, Bergada C (1976) Effect of hCG on the interstitial cells and androgen production in the immature rat testis. J Reprod Fertil 46: 279-282.

138 Chen YDI, Payne AH, Kelch RP (1976) FSH stimulation of Leydig cell function in the hypophysectomized immature rats. Proc Soc Exp Biol (NY) 153:473-475.

139 Chen YDI, Shaw MJ, Payne AH (1977) Steroid and FSH action on LH receptors and LH-sensitive testicular responsiveness during sexual maturation of the rat. Mol Cell Endocrinol 8:291-299.

140 Chipman JJ, Moore CJ, Marks JF, Fevre M, Segel T, Ramsay J, Boyar RM (1981) Interrelationship of plasma and urinary gonadotropins: correlation for 24 hours, for sleep/wake periods and for 3 hours after luteinizing hormone-releasing hormone stimulation. J Clin Endocrinol Metab 52:225-230.

141 Chopra IJ, Tulchinsky D (1974) Status of oestrogen-androgen balance in hyperthyroid men with Graves' disease. J Clin Endocrinol Metab 38:269-277.

142 Christensen AK, Mason NR (1965) Comparative ability of seminiferous tubules and interstitial tissue of rat testes to synthesize androgen from progesterone-4-^{14}C in vitro. Endocrinology 76:646-656.

143 Christensen AK, Peacock KC (1980) Increase in Leydig cell number in testes of adult rats treated chronically with an excess of human chorionic gonadotrophin. Biol Reprod 22:383-391.

144 Christiansen P (1975) Studies on the relationship between spermatogenesis and urinary levels of follicle-stimulating hormone and luteinizing hormone in oligospermic men. Acta Endocrinol 78:192-208.

145 Cigorraga SB, Dufau ML, Catt KJ (1978) Regulation of luteinizing hormone receptors and steroidogenesis in gonadotrophin-desensitized Leydig cells. J Biol Chem 253:4297-4304.

146 Cigorraga SB, Sorell S, Bator J, Catt KJ, Dufau ML (1980) Estrogen dependence of a gonadotropin-induced steroidogenic lesion in rat testicular Leydig cells. J Clin Invest 65:699-705.

147 Clayton RN, Catt KJ (1981) Gonadotropin-releasing hormone receptors: characterization, physiological regulation and relationship to reproductive function. Endocr Rev 2:186-209.

148 Clayton RN, Katikineni M, Chan V, Dufau ML, Catt KJ (1980) Direct inhibition of testicular function by gonadotropin-releasing hormone - mediation by specific gonadotropin-releasing hormone receptors in interstitial cells. Proc Natl Acad Sci USA 77: 4459-4463.

149 Clayton RN, Shakespear RA, Duncan JA, Marshall JC, Munson PJ, Rodbard D (1979) Radioiodinated non-degradable gonadotropin-releasing hormone analogs: new probes for the investigation of pituitary gonadotropin-releasing hormone receptors. Endocrinology 105:1369-1376.

150 Clegg EJ (1966) Pubertal growth in the Leydig cells and accessory reproductive organs of the rat. J Anat 100:369-379.

151 Clermont Y (1963) The cycles of the seminiferous epithelium in man. Am J Anat 112:35-51.

152 Clermont Y (1966) Renewal of spermatogonia in man. Am J Anat 118:509-524.

153 Clermont Y (1972) Kinetics of spermatogenesis in mammals. Physiol Rev 52:198-236.

154 Coble Jr YD, Kohler PO, Cargill CM, Ross GT (1969) Production rates and metabolic clearance rates of human follicle-stimulating hormone in premenopausal and postmenopausal women. J Clin Invest 48:359-363.

155 Comhaire F, Vermeulen A (1975) Plasma testosterone in patients with varicocoele and sexual inadequacy. J Clin Endocrinol Metab 40:824-829.

156 Comite F, Culter GB, Rivier J, Vale WW, Loriaux DL, Crowley WF (1981) Short-term treatment of idiopathic precocious puberty with a long-acting analogue of luteinizing hormone-releasing hormone. N Engl J Med 305:1546-1550.

157 Congote LF, Solomon S (1975) Testosterone stimulation of a rapidly labelled, low molecular weight RNA fraction in human hepatic erythroid cells in culture. Proc Natl Acad Sci USA 72:523-527.

158 Conn PM, Marian J, McMillian M, Stern J, Rogers D, Hamby M, Penna A, Grant E (1981) Gonadotropin-releasing hormone action in the pituitary: a three step mechanism. Endocr Rev 2:174-185.

159 Conn PM, Rogers DC, Sandhu FS (1979) Alteration of the intracellular calcium level stimulates gonadotropin release from cultured rat anterior pituitary cells. Endocrinology 105:1122-1127.

160 Connell GM, Eik-Nes KB (1968) Testosterone production by rabbit testis slices. Steroids 12:507-516.

161 Conte FA, Grumbach MM, Kaplan SL (1975) A diphasic pattern of gonadotropin secretion in patients with syndrome of gonadal dysgenesis. J Clin Endocrinol Metab 40:670-675.

162 Conte FA, Grumbach MM, Kaplan SL, Reiter EO (1980) Correlation of luteinizing hormone-releasing factor induced luteinizing hormone and follicle-stimulating hormone release from infancy to 19 years with the changing pattern of gonadotropin secretion in agonadal patients - relation to the restraint of puberty. J Clin Endocrinol Metab 50:163-168.

163 Cooke BA (1976) Correlation of protein kinase activation and testosterone production after stimulation of Leydig cells with luteinizing hormone. Biochem J 160:439-446.

164 Cooke BA, de Jong FH, van der Molen HJ, Rommerts FFG (1972) Endogenous testosterone concentrations in the rat testis interstitial tissue and seminiferous tubules during in vitro incubation. Nature (New Biol) 237:255-256.

165 Cooke BA, Janszen FHA, Clotscher WF, van der Molen HJ (1975) Effect of protein synthesis inhibitors on testosterone production in rat testis interstitial tissue and Leydig cell preparations. Biochem J 150: 413-418.

166 Cooke BA, Lindh LM, Janszen FHA (1977) Effect of lutropin on phosphorylation of endogenous proteins in testis Leydig cells. Biochem J 168:43-48.

167 Corker CS, Davidson DW (1978) Radioimmunoassay of testosterone in various biological fluids without chromatography. J Steroid Biochem 9:373-374.

168 Corley TP, Valk TW, Kelch RP, Marshall JC (1981) Estimation of GnRH pulse amplitude during pubertal development. Pediatr Res 15:157-162.

169 Corvol P, Bardin CW (1973) Species distribution of testosterone-binding globulin. Biol Reprod 8:277-282.

170 Corvol PL, Chrambach A, Rodbard D, Bardin CW (1971) Physical properties and binding capacity of testosterone-estradiol binding globulin in human plasma, determined by polyacrylamide gel electrophoresis. J Biol Chem 246:3435-3443.

171 Coscia AM, Fleischer N, Besch PK, Brown LP, Desiderio D (1974) The effect of synthetic luteinizing hormone-releasing factor on plasma LH levels in pituitary disease. J Clin Endocrinol Metab 38:83-88.

172 Courot M, Hochereau-de-Reviers M-H, Monet-Kuntz C, Locatelli A, Pisselet C, Blanc MR, Dacheux JL (1979) Endocrinology of spermatogenesis in the hypophysectomized ram. J Reprod Fertil (Suppl) 26: 165-173.

173 Crépy O, Dray F, Sebraoum Y (1967) Rôle des hormones thyroidennes dans les interactions entre la testosterone et les proteines seriques. C R Acad Sci (Paris) 2641-2657.

174 Critchlow V, Bar-Sela ME (1966) Control of the onset of puberty. In: Neuroendocrinology. Eds: L Martini, WF Ganong. Academic Press, New York. Vol II, pp101-162.

175 Crowley WF, McArthur JW (1980) Stimulation of the normal menstrual cycle in Kallmann's syndrome by pulsatile administration of luteinizing hormone-releasing hormone (LHRH). J Clin Endocrinol Metab 51:173-175.

176 Cunningham GR, Huckins C (1979) Persistence of complete spermatogenesis in the presence of low intratesticular concentrations of testosterone. Endocrinology 105:177-186.

177 Cutler GB, Loriaux DL (1980) Adrenarche and its relationship to the onset of puberty. Fed Proc 39: 2384-2390.

178 Damassa DA, Kobashigawa D, Smith ER, Davidson JM (1976) Negative feedback control of LH by testosterone: a quantitative study in male rats. Endocrinology 99: 736-742.

179 Damber JE, Bergh A (1980) Decreased testicular response to acute LH stimulation and increased intratesticular concentration of oestradiol-17β in the abdominal testes in cryptorchid rats. Acta Endocrinol 95:416-421.

180 Damber JE, Bergh A, Janson PO (1978) Testicular blood flow and testosterone concentrations in the spermatic venous blood in rat with experimental cryptorchidism. Acta Endocrinol 88:611-618.

181 Daughaday WH (1958) Binding of corticosteroids by plasma proteins III. The binding of corticosteroid and related hormones by human plasma and plasma protein fractions as measured by equilibrium dialysis. J Clin Invest 37:511-518.

182 David KI, Dingemanse E, Freud J, Laquer E (1935) Uber krystallinisches männliches Hormon aus Hoden (Testosteron) Wirsamer als aus Harn oder aus Cholesterin bereitetes Androsteron. Z Physiol Chem 233:281-282.

183 Davidson JM (1969) Feedback control of gonadotropin secretion. In: Frontiers in Neuroendocrinology. Eds: WF Ganong, L Martini. Oxford University Press, New York, p343-388.

184 Davidson JM (1972) Hormones and reproductive behaviour. In: Hormones and Behaviour. Ed: S Levine. Academic Press, New York, pp63-103.

185 Davidson JM, Damassa DA, Smith ER, Cheung C (1976) Feedback control of gonadotropin secretion in the male rat. In: Regulatory Mechanisms of Male Reproductive Physiology. Eds: CH Spilman, TJ Lobl, KT Kirton. Excerpta Medica, Amsterdam, pp151-168.

186 Davies IJ, Naftolin F, Ryan KJ, Siu J (1975) A specific high affinity, limited capacity oestrogen binding component in the cytosol of human foetal pituitary and brain tissues. J Clin Endocrinol Metab 40:909-912.

187 Davies P, Griffiths K (1974) Effects of α-amanitin on the stimulation of prostatic ribonucleic acid polymerase by prostatic steroid-receptor complexes. Biochem J 140:565-567.

188 Davies RV, Main SJ, Setchell BP (1978) Inhibin: evidence for its existence, methods of bioassay and nature of the active material. Int J Androl (Suppl) 2:102-113.

189 Daw SF (1970) Age of boys' puberty in Leipzig 1724-49 as studied by J.S. Bach's choir members. Hum Biol 42:87-89.

190 Dean PDG, Exley D, Johnson MW (1971) Preparation of 17β-oestradiol-6-(O-carboxymethyl)-oxime-bovine serum albumin conjugate. Steroids 18:593-603.

191 Debeljuk L, Arimura A, Schally AV (1972) Effect of testosterone and estradiol on the LH-releasing hormone (LH-RH) in intact male rats. Endocrinology 90:1578-1581.

192 de Kretser DM (1967) Changes in the fine structure of the human testicular interstitial cells after treatment with human gonadotropins. Z Zellforsch 83: 344-358.

193 de Kretser DM (1979) Endocrinology of male infertility. Br Med Bull 35:187-192.

194 de Kretser DM, Burger HG, Dumpys R (1975a) Serum LH and FSH response in four-hour infusions of luteinizing hormone-releasing hormone in normal men. Sertoli cell only syndrome and Klinefelter's syndrome. J Clin Endocrinol Metab 41:876-886.

195 de Krester DM, Burger HG, Fortunes D, Hudson B, Long AR, Paulsen CA, Taft HP (1972) Hormonal, histological and chromosomal studies in adult males with testicular disorders. J Clin Endocrinol Metab 35: 392-401.

196 de Kretser DM, Burger HG, Hudson B (1974) The relationship between germinal cells and serum FSH levels in males with infertility. J Clin Endocrinol Metab 38:787-793.

197 de Kretser DM, Burger HG, Hudson B, Keogh EJ (1975b) The HCG stimulation test in men with testicular disorders. Clin Endocrinol 4:591-596.

198 de Kretser DM, Catt KJ, Burger HG, Smith DC (1969) Radioautographic studies on the localization of ^{125}I-labelled human luteinizing and growth hormone in immature male rats. J Endocrinol 43:105-111.

199 de Kretser DM, Sharpe RM, Swanston IA (1979) Alterations in steroidogenesis and human chorionic gonadotrophin binding in the cryptorchid rat testis. Endocrinology 105:135-138.

200 Demisch K, Magnett W, Neubaurer M, Schöffling K (1973) Studies about unconjugated androstenediol in human peripheral plasma. J Clin Endocrinol Metab 37: 129-134.

201 De Moor P, Steeno O, Heyns W (1968) Possible role or significance of protein-steroid binding in plasma. Ann Endocrinol (Paris) 29:119-122.

202 Demoulin A, Koulischer L, Hustin J, Hazee-Hagelstein MT, Lambotte R, Franchimont P (1979) Organ culture of mammalian testis III. Inhibin secretion. Horm Res 10:177-190.

203 de Peretti E, Forest MG (1976) Unconjugated dehydroepiandrosterone plasma levels in normal subjects from birth to adolescence in human: the use of a sensitive radioimmunoassay. J Clin Endocrinol Metab 43:982-991.

204 de Peretti E, Forest MG (1978) Pattern of plasma dehydroepiandrosterone sulfate levels in human from birth to adulthood: evidence for testicular production. J Clin Endocrinol Metab 47:572-577.

205 Desjardins C (1978) Endocrine regulation of reproductive development and function in the male. J Anim Sci 47 (Suppl 2):56-77.

206 Dhom G (1973) The prepubertal and pubertal growth of the adrenal (adrenarche). Beitr Path Bd 150:357-377.

207 Dickerman Z, Prager-Levine R, Laron Z (1976) Response of plasma LH and FSH to synthetic LH-RH in children at various pubertal stages. Am J Dis Child 130: 634-638.

208 Diczfalusy E, Troen P (1961) Endocrine functions of the human placenta. Vitam Horm 19:229-311.

209 Dierschke DJ, Bhattacharya AN, Atkinson LE, Knobil E (1970) Circhoral oscillations of plasma LH levels on the ovariectomized rhesus monkey. Endocrinology 87: 850-853.

210 Dierschke DJ, Karsch FJ, Weick RF, Weiss G, Hotchkiss J, Knobil E (1974) Hypothalamic-pituitary regulation of puberty: feedback control of gonadotropin secretion in the Rhesus monkey. In: Control of the Onset of Puberty. Eds: MM Grumbach, GD Grave, FE Mayer. John Wiley & Sons, New York, pp104-114.

211 Dionne FT, Dube JY, Lesage RL, Tremblay RR (1979) In vivo androgen binding in rat skeletal and perineal muscles. Acta Endocrinol 91:362-372.

212 Dixon WJ, Brown MB (eds)(1979) Biomedical Computer Programmes, P series. University of California Press, Berkeley, pp581-598.

213 Döhler KD, Wuttke W (1975) Changes with age in levels of serum gonadotrophins, prolactin and gonadal steroids in prepubertal male and female rats. Endocrinology 97:899-907.

214 Donovan BT (1963) The timing of puberty. Sci Basis Med Annu Rev, pp53-75.

215 Donovan BT, van der Werff ten Bosch JJ (1959) The hypothalamus and sexual maturation in the rat. J Physiol 147:78-92.

216 Donovan BT, van der Werff ten Bosch (1965) Physiology of puberty, 1st Ed. Arnold, London.

217 Dorfman RI, Shipley RA (1956) Androgens. Wiley, New York, pp3-9.

218 Dorrington JH, Armstrong DT (1975) Follicle-stimulating hormone stimulates estradiol-17β synthesis in cultured Sertoli cells. Proc Natl Acad Sci USA 72:2677-2681.

219 Dorrington JH, Armstrong DT (1979) Effects of FSH on gonadal functions. Recent Prog Horm Res 35:301-342.

220 Dorrington JH, Fritz IB (1975) Cellular localization of 5α reductase and 3α hydroxysteroid dehydrogenase in the seminiferous tubule of the rat testis. Endocrinology 96:879-889.

221 Drouva SV, Gallo RV (1976) Catecholamine involvement in episodic luteinizing hormone release in adult ovariectomized rats. Endocrinology 99:651-658.

222 Ducharme J-R, Forest MG, de Peretti E, Sempe M, Collu R, Bertrand J (1976) Plasma adrenal and gonadal sex steroids in human pubertal development. J Clin Endocrinol Metab 42:468-476.

223 Dufau ML, Beitins IZ, McArthur JW, Catt KJ (1977) Bioassay of serum LH concentrations in normal and LH-RH stimulated human subjects. In: The Testis in Normal and Infertile Men. Eds: P Troen, HR Nankin. Raven Press, New York, pp309-325.

224 Dufau ML, Cigorraga SB, Baukal AJ, Sorrell SH, Bator JM, Neubauer JF, Catt KJ (1979) Androgen biosynthesis in Leydig cells after testicular desensitization by luteinizing hormone-releasing hormone and human chorionic gonadotrophin. Endocrinology 105:1314-1321.

225 Dufau ML, Hsueh AJ, Cigorraga S, Baukal AJ, Catt KJ (1978) Inhibition of Leydig cell function through hormonal regulatory mechanisms. Int J Androl (Suppl) 2:193-239.

226 Dufau ML, Tsuruhara T, Horner K, Podesta H, Catt KJ (1977) Intermediate role of adenosine 3'5' cyclic monophosphate and protein kinase during gonadotrophin induced steroidogenesis in testicular interstitial cells. Proc Natl Acad Sci USA 74:3419-3423.

227 Dunn TB (1949) Some observations on the normal and pathologic anatomy of the kidney of the mouse. J Natl Cancer Inst 9:285-301.

228 Dvoskin S (1944) Local maintenance of spermatogenesis by intracellularly implanted pellets of testosterone in hypophysectomized rats. Am J Anat 75:289-327.

229 Dym M, Cavicchia JC (1978) Functional morphology of the testis. Biol Reprod 18:1-15.

230 Dym M, Raj HGM, Lin YC, Chemes HE, Kotite NJ, Nayfeh SN, French FS (1979) Is FSH required for maintenance of spermatogenesis in adult rats? J Reprod Fertil (Suppl) 26:175-181.

231 Ehara YS, Yen SSC, Siler TM (1975) Serum prolactin levels during puberty. Am J Obstet Gynecol 121: 995-997.

232 Ehrenkranz JRL, Tamarkin L, Comite F, Johnsonbaugh RE, Bybee DE, Loriaux DL, Cutler Jr GB (1982) Daily rhythm of plasma melatonin in normal and precocious puberty. J Clin Endocrinol Metab 55:307-310.

233 Eik-Nes KB (1970) Synthesis and secretion of androstenedione and testosterone. In: The Androgens of the Testis. Ed: KB Eik-Nes. Marcel Dekker Inc., New York, pp10-14.

234 Eik-Nes KB (1971) Production and secretion of testicular steroids. Recent Prog Hom Res 27:517-535.

235 Ekins RP (1960) The estimation of thyroxine in human plasma by an electrophoretic technique. Clin Chim Acta 5:453-459.

236 Eliasson R (1975) Analysis of semen. In: Progress in Infertility, Ed. 2. Eds: SJ Behrman, RW Kistner. Little, Brown & Co, Boston, pp691-713.

237 Elkington JSH, Sanborn BM, Steinberger E (1975) The effect of testosterone proprionate on the concentration of testicular and epididymal androgen binding protein. Mol Cell Endocrinol 2:157-170.

238 El Makhzangy ME, Wynn V, Lawrence DM (1979) Sex hormone binding globulin capacity as an index of oestrogenicity or androgenicity in women on oral contraceptive steroids. Clin Endocrinol 10:39-45.

239 El Safoury, Bartke A (1974) Effects of follicle-stimulating hormone and luteinizing hormone on plasma testosterone levels in hypophysectomized and in intact immature and adult male rats. J Endocrinol 61: 193-198.

240 Erhardt AA, Grisanti GC, Meyer-Bahlburg HFL (1977) Prenatal exposure to medroxyprogesterone acetate (MPA) in girls. Psychoneuroendocrinology 2:391-398.

241 Eskay RL, Mical RS, Porter JC (1977) Relationship between luteinizing hormone releasing hormone concentration in hypophysial portal blood and luteinizing hormone release in intact, castrated and electrochemically-stimulated rats. Endocrinology 100: 263-270.

242 Everett JW (1954) Luteotropic function of autografts on the rat hypophysis. Endocrinology 54:685-690.

243 Everett JW (1977) The timing of ovulation. J Endocrinol 72:3P-13P.

244 Ewing L, Brown BL (1977) Testicular steroidogenesis. In: The Testis, Vol 4. Eds: AD Johnson, WR Gomes, NL Van Demark. Academic Press, New York, pp239-287.

245 Exley D, Johnson MW, Dean PDG (1971) Antisera highly specific for 17β-oestradiol. Steroids 18:605-620.

246 Faiman D, Ryan RJ (1967) Diurnal cycle in serum concentrations in follicle-stimulating hormone in men. Nature 215:857-859.

247 Faiman C, Winter JSD (1971) Diurnal cycles in plasma FSH, testosterone and cortisol in man. J Clin Endocrinol Metab 33:186-192.

248 Faiman C, Winter JD (1974) Gonadotropins and sex hormone patterns in puberty: clinical data. In: The Control of the Onset of Puberty. Eds: MM Grumbach, GD Grave, FE Mayer. John Wiley & Sons, New York, pp32-35.

249 Faiman C, Winter JSD, Reyes FI (1981) Endocrinology of the fetal testis. In: The Testis. Eds: H Burger, DM de Kretser. Raven Press, New York, pp81-105.

250 Fan DF, Oshima H, Troen B, Troen P (1974) Studies on the human testis IV. Testicular 20α-hydroxysteroid dehydrogenase and steroid 17α-hydroxylase. Biochim Biophys Acta 360:88-99.

251 Fang S, Anderson KM, Liao S (1969) Receptor proteins for androgens: on the role of specific proteins in selective retention of 17β-hydroxy-5α-androstan-3-one by rat ventral prostate in vivo and in vitro. J Biol Chem 244:6584-6595.

252 Fang S, Liao S (1971) Androgen receptors: steroid and tissue specific retention of a 17β-hydroxy-5α-androstan-3-one protein complex by the cell nuclei of ventrol prostate. J Biol Chem 246:16-24.

253 Fawcett DW (1975) Ultrastructure and function of the Sertoli cell. In: Handbook of Physiology, Section 7, Vol V. Eds: RO Greep, EB Astwood. American Physiological Society, Washington DC, pp21-56.

254 Fevold HL, Hishaw FL, Leonard SL (1931) The gonad stimulating and luteinizing hormones of the anterior lobe of the hypophysis. Am J Physiol 97:291-301.

255 Fevre M, Boyar RM, Rollag MD (1979) Dosage radio-immunologique de la mélatonine au cours du nycthemére chez le garcon pubere. Ann Endocrinol (Paris) 40: 555-556.

256 Ficher M, Steinberger E (1971) In vitro progesterone metabolism by rat testicular tissue at different stages of development. Acta Endocrinol 68:285-292.

257 Fiet J, Pham-Huu-Trung MT (1979) Radioimmunoassay of 17-hydroxyprogesterone: physiological variations and pathological values in man. J Steroid Biochem 10: 569-570.

258 Fink G (1979) Neuroendocrine control of gonadotrophin secretion. Br Med Bull 35:155-160.

259 Finkelstein JW, Kapen S, Weitzman ED, Hellman L, Boyar RM (1978) Twenty-four hour plasma prolactin patterns in prepubertal and adolescent boys. J Clin Endocrinol Metab 47:1123-1128.

260 Fiorelli G, Borrelli D, Forti G, Gonnelli P, Pazzagli M, Serio M (1976) Simultaneous determination of androstenedione, testosterone and 5α-dihydrotesto-sterone in human spermatic and peripheral venous plasma. J Steroid Biochem 7:113-116.

261 Fishman J (1976) The catechol estrogens. Neuroendocrinology 22:363-374.

262 Foldesy RG, Leatham JH (1980) Simultaneous measure-ments of testosterone and three 5α-reduced androgens in the venous effluent of immature rat testes in situ. Steroids 35:621-631.

263 Forest MG (1981) Control of the onset of puberty. In: Endocrinology of Human Infertility: New Aspects. Eds: C Ferrari, E Reschini. Academic Press, New York, pp265-306.

264 Forest MG, Bertrand J (1972) Studies of the protein binding of testosterone, androstenedione and dehydro-isoandrosterone in human plasma. Steroids 19:197-214.

265 Forest MG, Bertrand J (1975) Sexual steroids in neo-natal period. J Steroid Biochem 6:R24-R26.

266 Forest MG, de Peretti E, Bertrand J (1978) Develop-mental patterns of the plasma levels of testosterone, Δ^4-androstenedione, 17α-hydroxyprogesterone, dehydro-epiandrosterone and its sulphate in normal infants and prepubertal children. In: The Endocrine Function of the Human Adrenal Cortex. Eds: VHT James, M Serio, G Giusti, L Martini. Academic Press, New York, pp561-582.

267 Forest MG, Lecog A, Saez JM (1979) Kinetics of human chorionic gonadotropin-induced steroidogenic response of the human testis II. Plasma 17α hydroxyprogesterone, Δ^4 androstenedione, estrone and 17β-estradiol; evidence for the action of human chorionic gonadotropin on intermediate enzymes implicated in steroid biosynthesis. J Clin Endocrinol Metab 49:284-291.

268 Foster DL, Mickelson IH, Ryan KD, Coon GA, Drongowski RA, Holt JA (1978) Ontogeny of pulsatile luteinizing hormone and testosterone secretion in male lambs. Endocrinology 102:1137-1146.

269 Franchimont P, Chari S, Demoulin A (1975) Hypothalamus-pituitary-testis interaction. J Reprod Fertil 44:335-350.

270 Franchimont P, Chari S, Hazee-Hagelstein MT, Debruche ML, Duraiswami S (1977) Evidence for the existence of inhibin. In: The Testis in Normal and Infertile Men. Eds: P Troen, HR Nankin. Raven Press, New York, pp253-279.

271 Franchimont P, Demoulin A, Bourguignon JP (1975) Clinical use of LH-RH test as a diagnostic tool. Horm Res 6:177-191.

272 Franchimont P, Demoulin A, Verstraelen-Proyard J, Hazee-Hagelstein MT, Walton JS, Waites GMH (1978) Nature and mechanism of action of inhibin: perspective in regulation of male fertility. Int J Androl (Suppl) 2:69-80.

273 Franchimont P, Millet D, Vendreley E, Letawe J, Legros JJ, Netter A (1972) Relationship between spermatogenesis and serum gonadotrophin levels in azoospermia and oligospermia. J Clin Endocrinol Metab 34:1003-1008.

274 Franchimont P, Verstralen-Proyard J, Hazee-Hagelstein MT, Renard C, Demoulin A, Bourgignon JP, Hustin J (1979) Inhibin: from concept to reality. Vitam Horm 37:243-302.

275 Franks S, Jacobs HS, Martin N, Nabarro JDN (1978) Hyperprolactinaemia and impotence. Clin Endocrinol 8:277-287.

276 Franz C, Watson D, Longcope C (1979) Oestrone sulphate and dehydroepiandrosterone sulphate in normal subjects and men with cirrhosis. Steroids 34:563-573.

277 Fraser HM (1981) Luteinizing hormone-releasing hormone and fertility control. Oxford Rev Reprod Biol 3:1-48.

278 Frasier SD, Gatford F, Horton R (1969) Plasma androgens in childhood and adolescence. J Clin Endocrinol Metab 29:1404-1408.

279 Frasier SD, Horton R (1966) Androgens in the peripheral plasma of prepubertal children and adults. Steroids 8:777-783.

280 Freund M (1962) Interrelationships among the characteristics of human semen and factors affecting semen specimen quality. J Reprod Fertil 4:143-159.

281 Freund M (1963) Effect of frequency of emission on semen output and an estimate of daily sperm production in man. J Reprod Fertil 6:269-286.

282 Frisch RE, McArthur JW (1974) Menstrual cycle: fatness as a determinant of minimum weight for height necessary for their maintenance on onset. Science 185:959-961.

283 Frisch RE, Revelle R (1970) Height and weight at menarche and a hypothesis of critical body weight and adolescent events. Science 169:397-399.

284 Frisch RE, Revelle R (1971) Height and weight at menarche and a hypothesis of menarche. Arch Dis Child 46:695-701.

285 Fritz IB (1978) Site of action of androgens and follicle stimulating hormone on cells of the seminiferous tubule. In: Biochemical action of hormones. Ed: G Litwak. Academic Press, New York, 5:249-281.

286 Frowein J, Engel W (1974) Constituitivity of the HCG receptor protein in the testis of rat and man. Nature 249:377-379.

287 Frowein J, Engel W (1975) Binding of human chorionic gonadotropin by rat testis: effect of sexual maturation, cryptorchidism and hypophysectomy. J Endocrinol 64:59-66.

288 Fuxe K, Eneroth P, Gustafsson J-A, Löfström A, Skett P (1977) Dopamine in the nucleus accumbens: preferential increase of DA turnover by rat prolactin. Brain Res 122:177-182.

289 Fuxe K, Hökfelt T (1969) Catecholamines in the hypothalamus and the pituitary gland. In: Frontiers in Neuroendocrinology Vol 1. Eds: WF Ganong, L Martini. Oxford University Press, London, pp47-96.

290 Fuxe K, Hökfelt T, Löfström A, Johansson O, Agnati L, Everitt B, Goldstein M, Jeffcoate S, White N, Eneroth P, Gustafsson J-A, Skett P (1976) On the role of neurotransmitters and hypothalamic hormones and their

interactions in hypothalamic and extra-hypothalamic control of pituitary function and sexual behaviour. In: Subcellular Mechanisms in Reproductive Neuroendocrinology, Eds: F Naftolin, KJ Ryan, IJ Davies. Elsevier Scientific Publications, Amsterdam, ppl93-246.

291 Fuxe K, Löfström A, Hökfelt T, Ferland L, Andersson K, Agnati L, Eneroth P, Gustafsson J-A, Skett P (1978) Influence of central catecholamines on LHRH-containing pathways. Clin Obstet Gynecol 5:251-269.

292 Gallagher TF, Yoshida K, Roffwarg H, Weitzman ED, Fukushima D, Hellman L (1973) ACTH and cortisol secretory patterns in man. J Clin Endocrinol Metab 36:1058-1068.

293 Gallo RV, Moberg G (1977) Serotonin-mediated inhibition of episodic luteinizing hormone release during electrical stimulation of the arcuate nucleus in ovariectomized rats. Endocrinology 100:945-954.

294 Gandy HM, Peterson RE (1968) Measurement of testosterone and 17 ketosteroids in plasma by the double isotope derivative technique. J Clin Endocrinol Metab 28:949-977.

295 Gay VL, Kerlan JT (1978) Serum LH and FSH following passive immunization against circulating testosterone in intact male rats and in orchidectomized rats bearing subcutaneous silastic implants of testosterone. Arch Androl 1:257-266.

296 Gay VL, Sheth NA (1972) Evidence for a periodic release of LH in castrated male and female rats. Endocrinology 90:158-162.

297 Geiger R, König W, Wissmann H, Geisen K, Enzmann F (1971) Synthesis and characterization of a decapeptide having LH-RH-FSH-RH activity. Biochem Biophys Res Commun 45:767-773.

298 Gibbs DM, Neill JD (1978) Dopamine levels in hypophysial stalk blood in the rat are sufficient to inhibit prolactin secretion in vivo. Endocrinology 102:1895-1900.

299 Giusti M, Bolognesi F, Mortara R, Mignone D, Grimaldi P, Giordano G (1979) Sleep-wake patterns and integrated values of luteinizing hormone, follicle stimulating hormone , prolactin, growth hormone and thyroid stimulating hormone in normal and cryptorchid pubertal patients. Clin Endocrinol 11:523-532.

300 Glass AR, Swerdloff RS (1980) Nutritional influences on sexual maturation in the rat. Fed Proc 39: 2360-2364.

301 Goldzieher JW, Dozier TS, Smith KD, Steinberger E (1976) Improving the diagnostic reliability of rapidly fluctuating plasma hormone levels by optimized multiple sampling techniques. J Clin Endocrinol Metab 43:824-830.

302 Goldzieher JW, Roberts IS (1952) Identification of oestrogen in the human testis. J Clin Endocrinol Metab 12:143-150.

303 Goodman RL, Karsch FJ (1981) The hypothalamic pulse generator: a key determinant of reproductive cycles in sheep. In: Biological Clocks in Seasonal Reproductive Cycles, Eds: BK & DE Follett. Wright & Sons, Bristol, pp223-236.

304 Gordon GG, Olivio J, Rafii F, Southren AL (1975) Conversion of androgen to oestrogens in cirrhosis of the liver. J Clin Endocrinol Metab 40:1018-1026.

305 Gorski RA (1971) Gonadal hormones and the perinatal development of neuroendocrine function. In: Frontiers in Neuroendocrinology, Eds: L Martini, WF Ganong. Oxford University Press, London, pp237-290.

306 Gorski RA (1974) Extrahypothalamic influences on gonadotropin regulation. In: The Control of the Onset of Puberty, Eds: MM Grumbach, GD Grave, FE Mayer. John Wiley & Sons, New York, pp182-212.

307 Goy RW, Resko JA (1972) Gonadal hormones and behaviour of normal and pseudohermaphroditic non-human female primates. Recent Prog Horm Res 28: 707-733.

308 Graaf R de (1972) Tractatus de virorum organis generationi inservientibus. In: Regnier de Graaf on the Human Reproductive Organs, translated by HD Jocelyn, BP Setchell. J Reprod Fertil (Suppl) 17: 1-76.

309 Grant G, Vale W, Rivier J (1973) Pituitary binding sites for (^{3}H)-labelled luteinizing hormone-releasing factor (LRF). Biochem Biophys Res Commun 50:771-778.

310 Green JD (1951) The comparative anatomy of the hypophysis, with special reference to its blood supply and innervation. Am J Anat 88:225-312.

311 Green JD, Harris GW (1947) The neurovascular link between the neurohypophysis and adenohypophysis. J Endocrinol 5:136-146.

312 Green JD, Harris GW (1949) Observations of the hypophysio-portal vessels of the living rat. J Physiol 108:359-361.

313 Greep RO, Fevold HL (1937) The spermatogenic and secretory function of the gonads of hypophysectomized adult rats treated with pituitary FSH and LH. Endocrinology 21:611-618.

314 Greep RO, Fevold HL, Hishaw FL (1936) Effects of two hypophyseal gonadotropic hormones on the reproductive system of the male rat. Anat Rec 65:261-272.

315 Greep RO, Jones IC (1950) Steroid control of pituitary function. Recent Prog Horm Res 5:197-261.

316 Grodin JM, Siitteri PK, MacDonald PC (1973) Source of estrogen production in the post-menopausal woman. J Clin Endocrinol Metab 36:207-214.

317 Grumbach MM (1980) The neuroendocrinology of puberty. In: Neuroendocrinology: the Interrelationships of the body's two major integrative systems - in normal physiology and in clinical diseases. Eds: DT Krieger, JC Hughes. Sinauer Assoc Inc, Sunderland MA, pp249-258.

318 Grumbach MM, Richards GE, Conte F, Kaplan SL (1978) Clinical disorders of adrenal function and puberty: an assessment of the role of the adrenal cortex in normal and abnormal puberty in man and evidence for an ACTH-like pituitary adrenal androgen stimulating hormone. In: The Endocrine Function of Adrenal Cortex, Eds: VHT James, M Serio, G Giusti, L Martini. Academic Press, London, pp583-612.

319 Grumbach MM, Roth JC, Kaplan SL, Kelch RP (1974) Hypothalamic-pituitary regulation of puberty: Evidence and concepts derived from clinical research. In: The Control of Onset of Puberty, Eds: MM Grumbach, GD Grave, FE Mayer. John Wiley & Sons, New York, ppll5-166.

320 Guay AT, Tuthill RJ, Woolf PD (1977) Germinal cell aplasia: response of luteinizing hormone (LH), follicle-stimulating hormone (FSH) and testosterone to LH/FSH-releasing hormone with histopathologic correlation. Fertil Steril 28:642-649.

321 Gupta D, Rager K, Attanasio A, Klemm W, Eichner M (1975) Sex steroid hormones during multiphase pubertal developments. J Steroid Biochem 6:859-868.

322 Gustafsson J-A, Mode A, Norstedt G, Hökfelt T, Sonnenschein C, Eneroth P, Skett P (1980) The hypothalamo-pituitary-liver axis, a new hormonal system in control of hepatic steroid and drug metabolism. In: Biochemical Actions of Hormones, Ed: G Litwak. Academic Press, New York, Vol 7, pp47-90.

323 Gustafsson J-A, Pousette A (1974) Properties of nuclear 5 alpha-reductase in rat liver. Biochemistry 13:875-881.

324 Gustafsson J-A, Stenberg A (1974) Irreversible androgenic programming at birth of microsomal and soluble rat liver enzymes active on androstene-3,17-dione and 5-alpha-androstane-3 alpha-17 beta-diol. J Biol Chem 249:711-718.

325 Guyda HJ (1975) Heterogeneity of human growth hormone and prolactin secreted in vitro: immunoassay and radioreceptor assay correlations. J Clin Endocrinol Metab 41:953-967.

326 Guyda HJ, Friesen HG (1973) Serum prolactin levels in humans from birth to adult life. Pediatr Res 7:534-540.

327 Hafiez AA, Philpott JE, Bartke A (1971) The role of prolactin in the regulation of testicular function: the effect of prolactin and luteinizing hormone on 3β-hydroxysteroid dehydrogenase activity in the testes of mice and rats. J Endocrinol 50:619-623.

328 Halasz B (1972) Hypothalamic mechanisms controlling pituitary function. Prog Brain Res 38:97-118.

329 Hall PF (1970) Endocrinology of the testis. In: The Testis, Vol 2. Eds: AD Johnson, WR Gomes, NL Van Demark. Academic Press, New York, pp1-71.

330 Hall PF, Charponnier C, Nakamura M, Gabbiani G (1979) The role of microfilaments in the response of Leydig cells to luteinizing hormone. J Steroid Biochem 11:1361-1366.

331 Hall PF, Irby DC, de Kretser DM (1969) Conversion of cholesterol to androgens by rat testes: comparison of interstitial cells and seminiferous tubules. Endocrinology 84:488-496.

332 Hall PF, Shikita M (1974) The role of cytochrome P-450 in the side-chain cleavage of cholesterol. In: Gonadotropins and Gonadal Function, Ed: NR Moudgal. Academic Press, New York, pp403-410.

333 Hammond GL, Ruokonen A, Kontturi M, Kaskela E, Vikko R (1977) The simultaneous radioimmunoassay of seven steroids in human spermatic and peripheral. J Clin Endocrinol Metab 45:16-24.

334 Hansson V, Weddington SC, McLean WS, Smith AA, Nayfeh SN, French FS, Ritzen EM (1975) Regulation of seminiferous tubular function by FSH and androgen. J Reprod Fertil 44:363-375.

335 Haour F, Saez JM (1978) Leydig cell responsiveness to LH-hCG stimulation: mechanisms of hCG- and steroid-induced refractoriness. In: Structure and Function of the Gonadotropins, Ed: KW McKerns. Plenum Press, New York, pp497-516.

336 Harris GW (1948) Electrical stimulation of the hypothalamus and the mechanism of neural control of the adenohypophysis. J Physiol 107:418-429.

337 Harris GW, Jacobsohn D (1952) Functional grafts of the anterior pituitary gland. Proc R Soc Lond (Biol) 139:263-276.

338 Haseltine FP, Ohno S (1981) Mechanism of gonadal differentiation. Science 211:1272-1278.

339 Hashimoto T, Miyai K, Onishi T, Matsumoto K, Kumahara Y (1975) Comparison of short and long term treatment with synthetic LHRH and clomiphene citrate in male hypothalamic hypogonadism. J Clin Endocrinol Metab 41:905-910.

340 Hawkins RA, Oakey RE (1974) Estimation of oestrone sulphate, oestradiol 17β and oestrone in peripheral plasma: concentrations during the menstrual cycle and in men. J Endocrinol 60:3-17.

341 Hayward JN (1977) Functional and morphological aspects of hypothalamic neurons. Physiol Rev 57: 574-658.

342 Heape W (1905) Ovulation and degeneration of ova in the rabbit. Proc R Soc Lond (Biol) 76:260-268.

343 Heller CG, Clermont Y (1964) Kinetics of the germinal epithelium in man. Recent Prog Horm Res 20:545-575.

344 Heller CG, Leach DR (1971) Quantification of Leydig cells and measurement of Leydig-cell size following administration of human chorionic gonadotrophin to normal men. J Reprod Fertil 25:185-192.

345 Hembree WC, Bardin CW, Lipsett MB (1969) A study of estrogen metabolic clearance rates and transfer factor. J Clin Invest 48:1809-1819.

346 Hertwig O (1875) Beitrage zur Kemitniss der Beldung, Befruchtung und Teilung des tierischen Eiex. Morphol Jahrbuch (Leipzig) p347.

347 Hodgen GD, Sherins RJ (1973) Enzymes as markers of testicular growth and development in the rat. Endocrinology 93:985-989.

348 Hodges JK (1980) Regulation of oestrogen-induced LH release in male and female marmoset monkey (Callithrux jacchus). J Reprod Fertil 60:389-398.

349 Hoff JD, Lasley BL, Wang CF, Yen SSC (1977) The two pools of pituitary gonadotropin: regulation during the menstrual cycle. J Clin Endocrinol Metab 44: 302-312.

350 Honda Y, Takashashi K, Takashashi S, Azumi K, Irie M, Sakuma M, Tsushima T, Shizume K (1969) Growth hormone secretion during nocturnal sleep in normal subjects. J Clin Endocrinol Metab 29:20-29.

351 Hooker CW (1970) The intertubular tissue of the testis. In: The Testis Vol 1. Eds: AD Johnson, WR Gomes, NL Van Demark. Academic Press, New York, pp483-450.

352 Hopper BR, Yen SSC (1975) Circulating concentrations of dehydroepiandrosterone and dehydroepiandrosterone sulfate during puberty. J Clin Endocrinol Metab 40: 458-461.

353 Horton R, Tait JF (1966) Androstenedione production and interconversion rates measured in peripheral blood and studies on the possible site of its conversion to testosterone. J Clin Invest 45: 301-313.

354 Horton R, Tait JF (1967) In vivo conversion of dehydroisoandrosterone to plasma androstenedione and testosterone in man. J Clin Endocrinol Metab 27: 79-88.

355 Hotchkiss RS (1942) Testicular biopsy in the diagnosis and treatment of sterility in the male. Bull NY Acad Med 18:600-605.

356 Hotchkiss RS, Brunner EK, Grenley P (1938) Semen analyses of 200 fertile men. Am J Med Sci 196: 362-384.

357 Hsu A-F, Stratico D, Hosaka M, Troen P (1978) Studies of the human testis. Properties of human chorionic gonadotropin receptor in adult testis and relation to intratesticular testosterone concentration. J Clin Endocrinol Metab 47:529-536.

358 Hsueh AJW, Dufau ML, Catt KJ (1976) Regulation of LH-receptors in testicular interstitial cells by gonadotropin. Biochem Biophys Res Commun 72: 1145-1152.

359 Hsueh AJW, Dufau ML, Catt KJ (1977) Gonadotropin-induced regulation of luteinizing hormone receptors and desensitization of testicular 3',5'-cyclic AMP

and testosterone responses. Proc Natl Acad Sci USA 74:592-595.

360 Hsueh AJW, Dufau ML, Catt KJ (1978) Direct inhibitory effect of oestrogen on Leydig cell functions of hypophysectomized rats. Endocrinology 103:1096-1102.

361 Hsueh AJW, Erickson GF (1979) Extra-pituitary inhibition of testicular function by luteinizing hormone releasing hormone. Nature 281:66-67.

362 Hsueh AJW, Jones PBC (1981) Extrapituitary actions of gonadotropin-releasing hormone. Endocr Rev 2: 437-461.

363 Hudson B, Baker HWG, Eddie LW, Higginson RE, Burger HG, de Kretser DM, Dobos M, Lee VWK (1979) Bioassays for inhibin: a critical review. J Reprod Fertil (Suppl) 26:17-29.

364 Hudson B, Burger HG, de Kretser DM (1970) Testosterone plasma levels in normal and pathological conditions. Adv Exp Med Biol 10:423-437.

365 Hudson B, Coghlan JP, Dulmanis JP (1967) Testicular function in man. Ciba Foundation Colloquia in Endocrinology (Endocrinology of the Testis) 16: 140-155.

366 Huhtaniemi IT, Katikineni M, Chan V, Catt KJ (1981) Gonadotropin-induced positive regulation of testicular luteinizing hormone receptors. Endocrinology 108:58-65.

367 Huhtaniemi I, Leinonen P, Hammond GL, Vikko R (1980) Effect of oestrogen treatment on testicular LH/hCG receptors and endogenous steroids in prostatic cancer patients. Clin Endocrinol 13:561-568.

368 Hunter WM, Bennie JG (1979) Reduction of non-specific serum responses in human pituitary gonadotrophin radioimmunoassay. J Endocrinol 80:59-68.

369 Hunter WM, Edmond P, Watson GS, Maclean N (1974) Plasma LH and FSH levels in subfertile men. J Clin Endocrinol Metab 39:740-749.

370 Huseman CA, Kelch RP (1978) Gonadotropin responses and metabolism of synthetic gonadotropin-releasing hormone (GnRH) during constant infusion of GnRH in men and boys with delayed adolescence. J Clin Endocrinol Metab 47:1325-1331.

371 Huseman CA, Kugler JA, Schneider IG (1980) Mechanism of dopaminergic suppression of gonadotropin secretion in men. J Clin Endocrinol Metab 51:209-214.

372 Hwang P, Guyda H, Friesen H (1971) A radioimmunoassay for human prolactin. Proc Natl Acad Sci USA 68:1902-1906.

373 Illig R, Bambach M, Pluznik S, Zachmann M, Prader A (1973) Die Wirkung von synthetischem LH-RH auf die Freisetzung von LH und FSH bei Kindern und Jugendlichen. Schweiz Med Wochenschr 103:840-842.

374 Imperato-McGinley J, Guerrero L, Gautier T, Peterson RE (1974) Steroid 5α-reductase deficiency in man: an inherited form of male psuedohermaphroditism. Science 186:1213-1215.

375 Imperato-McGinley J, Peterson RE (1976) Male pseudohermaphrodite: the complexities of male phenotypic development. Am J Med 61:251-272.

376 Inano H, Heri Y, Tamaoki B (1967) Effect of age on testicular enzymes related to steroid bioconversion. Ciba Foundation Colloquia in Endocrinology 16: 105-117.

377 Irvine WJ, Toft AD, Wilson KS, Fraser R, Wilson A, Young J, Hunter WM, Ismail AAA, Burger PE (1974) The effect of synthetic corticotrophin analogues on adrenocortial, anterior pituitary and testicular function. J Clin Endocrinol Metab 39:522-529.

378 Isurgi K, Wakabayashi K, Fukutani K, Takayasu H, Tamaoki B, Oakaka M (1973) Responses of serum luteinizing hormone and follicle stimulating hormone levels to synthetic luteinizing-releasing hormone (LHRH) in various forms of testicular disorders. J Clin Endocrinol Metab 37:533-539.

379 Ito T, Horton R (1971) The source of plasma dihydrotestosterone in man. J Clin Invest 50:1621-1627.

380 Jacobson RI, Seyler Jr LE, Tamborlane WV, Gertner JM, Genel M (1979) Pulsatile subcutaneous nocturnal administration of GnRH by portable infusion pump in hypogonadotrophic hypogonadism: initiation of gonadotrophin responsiveness. J Clin Endocrinol Metab 49: 652-654.

381 Janszen FHA, Cooke BA, van Driel MJA, van der Molen HJ (1976) Purification and characterization of Leydig cells from rat testes. J Endocrinol 70: 345-359.

382 Janszen FHA, Cooke BA, van Driel MJA, van der Molen HJ (1978) The effect of lutropin on specific protein synthesis in tumour Leydig cells and Leydig cells from immature rats. Biochem J 172:147-153.

383 Jeffcoate SL, Holland DT, White N, Fraser HM, Gunn A, Crighton DB, Foster JP, Griffiths EC, Hooper KC, Sharp PJ (1975) The radioimmunoassay of hypothalamic hormones (TRH and LH-RH) and related immunoreactive peptides in biological fluids. In: Hypothalamic Hormones. Eds: M Motta, PG Crosignani, L Martini. Academic Press, New York, pp279-297.

384 Job JC, Chaussain JL, Garnier PE (1977) The use of luteinizing-hormone-releasing hormone in pediatric patients. Horm Res 8:171-187.

385 Job JC, Garnier PE, Chaussain JL, Binet E, Schlumberger AM, Toublanc JE (1976) Effect of synthetic LH-RH on the release of gonadotropins in hypophysio-gonadal disorders of children and adolescents VII. Constitutional delay of puberty in males. J Pediatr 88:494-498.

386 Job JC, Garnier PE, Chaussain JL, Milhaud G (1972) Elevation of LH and FSH after LHRH injection in normal children and in patients with disorders of puberty. J Clin Endocrinol Metab 35:473-476.

387 Johanson AJ, Guyda H, Light C, Migeon CJ, Blizzard RM (1969) Serum luteinizing hormone by radioimmunoassay in normal children. J Pediatr 74:416-424.

388 Johansson KNG, Currie BL, Folkers K, Bowers CY (1973) Biosynthesis of the luteinizing hormone-releasing hormone in mitochondrial preparations and by a possible pantetheine-template mechanism. Biochem Biophys Res Commun 50:8-13.

389 Johnsen SG (1970a) Testicular biopsy score count - A method for registration of spermatogenesis in human testes: normal values and results in 335 hypogonadal males. Hormones 1:1-25.

390 Johnsen SG (1970b) Investigations into the feed-back mechanism between spermatogenesis and gonadotropin level in man. In: The human testis. Eds: Rosemberg, CA Paulsen. Plenum Press, New York, pp231-248.

391 Johnson BH, Ewing LL (1971) Follicle-stimulating hormone and the regulation of testosterone secretion in rabbit testes. Science 173:635-637.

392 Johnson DC, Mallampati RS (1975) Serum luteinizing hormone and follicle-stimulating hormone concentrations in immature female rats treated with multiple injections and various amounts of luteinizing hormone releasing hormone. J Endocrinol 66:13-20.

393 Jones TM, Fang VS, Landau RL, Rosenfeld R (1978) Direct inhibition of Leydig cell function by oestradiol. J Clin Endocrinol Metab 47:1368-1373.

394 de Jong FH (1980b) Unpublished observations quoted in Setchell (1980b).

395 de Jong FH, Hey AH, van der Molen HJ (1974) Oestradiol 17β and testosterone in rat testis tissue: effects of gonadotrophin localization and production in vitro. J Endocrinol 60:409-419.

396 de Jong FH, Sharpe RM (1977) Gonadotrophins, testosterone and spermatogenesis in neonatally irradiated male rats: evidence for a role of the Sertoli cell in follicle-stimulating hormone feedback. J Endocrinol 75:209-219.

397 de Jong FH, Welschen R, Hermans WP, Smith SD, van der Molen HJ (1978) Effects of testicular and ovarian inhibin-like activity, using in vitro and in vivo systems. Int J Androl (Suppl) 2:125-138.

398 de Jong FH, Welschen R, Hermans WP, Smith SD, van der Molen HJ (1979) Effects of factors from ovarian follicular fluid and Sertoli cell culture medium on in vivo and in vitro release of pituitary gonadotrophins in the rat: an evaluation of systems for the assay of inhibin. J Reprod Fertil (Suppl) 26:47-59.

399 Josso N, Picard JY, Tran D (1977) The antimullerian hormone. Recent Prog Horm Res 33:117-167.

400 Jost A (1953) Problems of fetal endocrinology: the gonadal and hypophyseal hormones. Recent Prog Horm Res 8:379-418.

401 Jost A (1970) Hormonal factors in the sex differentiation of the mammalian foetus. Philos Trans R Soc Lond (Biol) 259:119-130.

402 Jost A (1972) A new look at the mechanisms controlling sex differentiation in mammals. Johns Hopkins Med J 130:38-53.

403 Jouvet M (1969) Biogenic amines and the states of sleep. Science 163:32-41.

404 Judd HL (1979) Biorhythms of gonadotropins and testicular hormone secretion. In: Endocrine Rhythms, Ed: DT Krieger. Raven Press, New York, pp299-324.

405 Judd HL, Parker DC, Rakoff JS, Hopper BR, Yen SSC (1974a) Elucidation of mechanisms of the nocturnal rise of testosterone in men. J Clin Endocrinol Metab 38-134-141.

406 Judd SJ, Rakoff JS, Yen SSC (1978) Inhibition of gonadotropin and prolactin release by dopamine: effect of endogenous estradiol levels. J Clin Endocrinol Metab 47:494-498.

407 Judd HL, Rebar R, Vandenberg G, Yen SSC (1974b) Effect of luteinizing hormone-releasing factor on Leydig cell function. J Clin Endocrinol Metab 38: 8-13.

408 Kalla NR, Nisula BC, Menard R, Loriaux DL (1980) The effect of estradiol on testicular testosterone biosynthesis. Endocrinology 106:35-39.

409 Kamberi IA, Mical RS, Porter JC (1971) Effects of anterior pituitary perfusion and intraventricular injection of catecholamines on prolactin release. Endocrinology 88:1012-1020.

410 Kandeel FR, Butt WR, Rudd BT, Lynch SS, London DR, Edwards RL (1979) Oestrogen modulation of gonadotrophin and prolactin release in women with anovulation and their responses to clomiphene. Clin Endocrinol 10:619-635.

411 Kapen S, Boyar R, Finkelstein J, Hellman L, Weitzman E (1974) Effect of sleep-wake cycle reversal on luteinizing hormone secretory pattern in puberty. J Clin Endocrinol Metab 39:293-299.

412 Karlberg P, Taranger J, Engström I, Lichenstein H, Svennberg-Redegren I (1976) Physical growth from birth to 16 years and longitudinal outcome of the study during the same age period. Acta Paediatr Scand (Suppl) 258:7-76.

413 Karsch FJ, Dierschke DJ, Knobil E (1973) Sexual differentiation of pituitary function: apparent difference between primates and rodents. Science 179:484-486.

414 Karsch FJ, Foster DL (1975) Sexual differentiation of the mechanism controlling the preovulatory discharge of luteinizing hormone in sheep. Endocrinology 97:373-379.

415 Kastin AJ, Schally AW, Schalch DS, Korenman SG, Miller MC, Gual C, Perez-Pasten E (1972) Characterization of the hormonal response to LHRH in prepubertal and adult subjects. Pediatr Res 6:481-486.

416 Katongole CB, Naftolin F, Short RV (1971) Relationship between blood levels of luteinizing hormone and testosterone in bulls and the effect of sexual stimulation. J Endocrinol 50:457-466.

417 Katzenellenbogen BS, Bhakoo HS, Ferguson ER, Lan NC, Tatee T, Tsai TL, Katzenellenbogen JA (1979) Estrogen and anti-estrogen action in reproductive tissues and tumours. Recent Prog Horm Res 35: 259-300.

418 Kelch RP, Clemens LE, Markovs M, Westhoff MH, Hawkins DW (1975) Metabolism and effects of synthetic gonadotropin-releasing hormone (GnRH) in children and adults. J Clin Endocrinol Metab 40:53-61.

419 Kelch RP, Grumbach MM, Kaplan SL (1972) Studies on the mechanism of puberty in man. In: Gonadotropins. Eds: BB Saxena, CG Beling, HM Gandy. John Wiley & Sons, New York, pp524-534.

420 Kelch RP, Jenner MR, Weinstein R, Kaplan S, Grumbach MM (1972) Oestradiol and testosterone secretion by human, simian and canine testes in males with hypogonadism and in male psuedohermaphrodites with the feminizing testes syndrome. J Clin Invest 51: 824-830.

421 Kelch RP, Kaplan SL, Grumbach MM (1973) Suppression of urinary and plasma follicle-stimulating hormone by exogenous estrogens in prepubertal and pubertal children. J Clin Invest 52:1122-1128.

422 Kelch RP, Lindholm UB, Jaffe RB (1971) Testosterone metabolism of target tissues 2. Human fetal and adult reproductive tissues, perineal skin and skeletal muscle. J Clin Endocrinol Metab 32:449-456.

423 Kelch RP, Markovs M, Huss J (1976) LH and FSH responsiveness to intravenous gonadotropin releasing hormone (GnRH) in children with hypothalamic or pituitary disorders: lack of effect of replacement therapy with human growth hormone. J Clin Endocrinol Metab 42:1104-1113.

424 Kennedy GC, Mitra J (1963) Body weight and food intake as initiating factors for puberty in the rat. J Physiol 166:408-418.

425 Keogh EJ, Lee VWK, Rennie GC, Burger HC, Hudson B, de Kretser DM (1976) Selective suppression of FSH by testicular extracts. Endocrinology 98:999-1004.

426 Kerkay J, Westphal U (1968) Steroid protein interactions XIX. Complex formation between α_1 acid-glycoprotein and steroid hormones. Biochim Biophys Acta 170:324-333.

427 Kerr JB, Rich KA, de Kretser DM (1979) Alterations of the fine structure and androgen secretion of the interstitial cells in the experimentally cryptorchid rat testis. Biol Reprod 20:409-422.

428 Ketelslegers JM, Hetzel WD, Sherins RJ, Catt KJ (1978) Developmental changes in testicular gonadotrophin receptors: plasma gonadotropins and plasma testosterone in the rat. Endocrinology 103:212-222.

429 Keye WR, Kelch RP, Nieswender GD, Jaffe RB (1973) Quantitation of endogenous and exogenous gonadotropin releasing hormone by radioimmunoassay. J Clin Endocrinol Metab 36:1263-1267.

430 Kinouchi T, Horton R (1974a) 3α-androstanediol kinetics in man. J Clin Endocrinol Metab 38:262-268.

431 Kinouchi T, Horton R (1974b) 3α-androstanediol kinetics in man. J Clin Invest 54:646-653.

432 Kinsey AC, Pomeroy WB, Martin CE (1948) Sexual behaviour in the human male. Saunders, Philadelphia.

433 Kirschner MA, Wider JA, Ross GT (1970) Leydig cell function in men with gonadotropin-producing testicular tumours. J Clin Endocrinol Metab 30: 504-511.

434 Khar A, Kunnert-Radek J, Jutisz M (1979) Involvement of microtubule and microfilament system in the GnRH induced release of gonadotropin by rat anterior pituitary cells in culture. FEBS Lett 104:410-414.

435 Kjessler B, Wide L (1973) FSH and KSH in relation to gametic output in 643 males. Acta Endocrinol 72: 243-256.

436 Kley HK, Wieglmann W, Nieschlag E, Solbach HG, Zimmermann H, Kruskemper HL (1974) LH and FSH and testosterone in plasma following LH-RH infusion: a combined test for pituitary and Leydig cell function. Acta Endocrinol 75:417-427.

437 Knobil E (1980) The neuroendocrine control of the menstrual cycle. Recent Prog Horm Res 36:53-88.

438 Knobil E (1981) Patterns of hypophysiotropic signals and gonadotropin secretion in the Rhesus monkey. Biol Reprod 24:44-49.

439 Knorr D, Bidlingmaier F, Butenandt O, Fendel H, Ehrt-Wehle R (1974) Plasma testosterone in male puberty I. Physiology of plasma testosterone. Acta Endocrinol 75:181-194.

440 Knorr DW, Vanha-Perttula T, Lipsett MB (1970) Structure and function of rat testis through pubescence. Endocrinology 86:1288-1304.

441 Kochakian CD (1975) Definition of androgens and protein anabolic steroids. Pharmacol Ther 1:149-177.

442 Kohler PO, Ross GT, Odell WD (1968) Metabolic clearance and production rates of human luteinizing hormone in pre and postmenopausal women. J Clin Invest 47:38-47.

443 Korenman SG, Lipsett MB (1964) Is testosterone glucuronoside uniquely derived from plasma testosterone? J Clin Invest 43:2125-2131.

444 Korner A (1965) Growth hormone control of biosynthesis of protein and ribonucleic acid. Recent Prog Horm Res 21:205-240.

445 Korth-Schultz S, Levine LS, New MI (1976a) Serum androgens in normal prepubertal and pubertal children with precocious puberty. J Clin Endocrinol Metab 42:117-124.

446 Korth-Schultz S, Levine LS, New MI (1976b) Dehydroepiandrosterone sulfate (D5) levels, a rapid test for abnormal adrenal androgen secretion. J Clin Endocrinol Metab 42:1005-1013.

447 Kraaipoel RJ, Degenhart HJ, Leferink JG (1975b) Incorporation of $H_2{}^{18}O$ into 20α,22R-di-OH-cholesterol: evidence for an epoxide-diol pathway in the adrenocortical cholesterol side-chain cleavage mechanism. FEBS Lett 57:294-300.

448 Kraaipoel RJ, Degenhart HJ, Leferink JG, van Beek V, de Leeuw-Boon H, Visser HKA (1975a) Pregnenolone formation from cholesterol in bovine adrenal cortex mitochondria: proposal of a new mechanism. FEBS Lett 50:204-209.

449 Krabbe S, Skakkebaek NE (1977) Gonadotropin-releasing hormone (LHRH) and human chorionic gonadotropin in the treatment of two boys with hypogonadotropic hypogonadism. Acta Paediatr Scand 66:361-365.

450 Krieger DT, Liotta AS, Brownstein MJ, Zimmerman EA (1980) ACTH, βlipotropin and related peptides in brain, pituitary and blood. Recent Prog Horm Res 36:277-344.

451 Krieger DT, Martin JB (1981) Brain peptides. N Engl J Med 304:876-885.

452 Krieger DT, Ossowski R, Fogel M, Allen W (1972) Lack of circadian periodicity of human serum FSH and LH levels. J Clin Endocrinol Metab 35:619-623.

453 Krulich L, Fawcett CP (1977) The hypothalamic hypophysiotropic hormones. In: Endocrine Physiology Vol II. Ed: SM McCann. University Park Press, Baltimore, pp35-92.

454 Kulin H, Bell P, Santen R, Ferber A (1975) Integration of pulsatile gonadotropin secretion by timed urinary measurements: an accurate and sensitive 3-hour test. J Clin Endocrinol Metab 40:783-789.

455 Kulin HE, Moore Jr RG, Santner SJ (1976) Circadian rhythms in gonadotropin excretion in prepubertal and pubertal children. J Clin Endocrinol Metab 42: 770-773.

456 Kulin HE, Reiter EO (1972) Gonadotrophin suppression by low dose oestrogen in man: evidence for differential effects upon FSH and LH. J Clin Endocrinol Metab 35:836-839.

457 Kurtz DT, Feigelson P (1977) Multihormonal induction of hepatic alpha 2U-globulin mRNA as measured by hybridization to complementary DNA. Proc Natl Acad Sci USA 74:4791-4795.

458 Laatikainen T, Laitinen EA, Vihko R (1969) Secretion of neutral steroid sulphate by the human testis. J Clin Endocrinol Metab 29:219-224.

459 Laatikainen T, Laitinen EA, Vihko R (1971) Secretion of free and sulphate-conjugates neutral steroids by the human testis. Effect of administration of human chorionic gonadotrophin. J Clin Endocrinol Metab 32: 59-64.

460 Labrie F, Beaulieu M, Caron MG, Raymond V (1978) The adenohypophyseal dopamine receptor. Specificity and modulation of its activity by estradiol. In: Progress in Prolactin Physiology and Pathology. Eds: C Robyn, M Harter. Elsevier North Holland/Biomedical Press, Amsterdam, pp121-136.

461 Labrie F, Pelletier G, Borgeat P, Drouin J, Ferland L, Belanger A (1976) Mode of action of hypothalamic regulatory hormones in the adenohypophysis. In: Frontiers in Neuroendocrinology. Eds: L Martini, WF Ganong. Raven Press, New York, pp63-94.

462 Labrie F, Pelletier G, Lemay A, Borgeat P, Barden N, Dupont A, Savary M, Coté J, Boucher R (1973) Control of protein synthesis in anterior pituitary gland. In: Protein Synthesis in Reproductive Tissue, 6th Symposium. Ed: E Diczfalusy, Geneva, pp301-340.

463 de Lacerda L, Kowarski A, Johanson AJ, Athanasiou R, Migeon CJ (1973) Integrated concentration and circadian variation of plasma testosterone in normal men. J Clin Endocrinol Metab 37:366-371.

464 Lacroix A, McKenna TJ, Rabinowitz D (1978) Sex steroid modulation of gonadotrophins in normal men and in androgen insensitivity syndrome. J Clin Endocrinol Metab 48:235-240.

465 Lacroix M, Smith FG, Fritz IB (1977) Secretion of plasminogen activator by Sertoli cell enriched cultures. Mol Cell Endocrinol 9:227-236.

466 de Lange WE, Sluiter WJ, Van Zanten AK, Doorhenbos H (1974) The effect of injection and infusion of LHRH on serum LH and FSH in normal males and boys with delayed puberty. Neth J Med 17:196-201.

467 Large DM, Anderson DC (1979) Twenty-four hour profiles of circulating androgens and oestrogens in male puberty with and without gynaecomastia. Clin Endocrinol 11:505-521.

468 Large DM, Anderson DC, Laing I (1980) Twenty-four hour profiles of serum prolactin during male puberty with and without gynaecomastia. Clin Endocrinol 12: 293-302.

469 Laron Z, Dickerman Z, Prager-Lewin R, Keret R, Halabe E (1975) Plasma LH and FSH response to LRH in boys with compensatory testicular hypertrophy. J Clin Endocrinol 40:977-981.

470 Lasnitzky I, Franklin HR (1972) The influence of serum on uptake, conversion and action of testosterone in rat prostate glands in organ culture. J Endocrinol 54:333-342.

471 Lawrence DM, Swyer GI (1974) Plasma testosterone and testosterone binding affinities in men with impotence, oligospermia, azoospermia and hypogonadism. Br Med J 1:349-351.

472 Leblond CP, Clermont Y (1952) Definition of the stages of the cycle of the seminiferous epithelium in the rat. Ann NY Acad Sci 55:548-574.

473 Lee PA, Gisriel DL (1980) Correlation of gonadotropins with unbound testosterone during puberty in males. Horm Res 12:130-136.

474 Lee PA, Jaffe RB, Midgley Jr AR (1974) Serum gonadotrophin, testosterone and prolactin concentrations throughout puberty in boys - a longitudinal study. J Clin Endocrinol Metab 39:664-672.

475 Lee PA, Midgeon CJ (1975) Puberty in boys: correlation of plasma levels of gonadotropin (LH, FSH), androgen (testosterone, androstenedione, dehydroepiandrosterone and its sulphate), estrogen (estrone and estradiol) and progestin (Progesterone, and 17-hydroxyprogesterone). J Clin Endocrinol Metab 41:556-562.

476 Lee VWK, Cumming IA, de Kretser DM, Findlay JK, Hudson B, Keogh EJ (1976) Regulation of gonadotrophin secretion in rams from birth to sexual maturity I. Plasma LH, FSH and testosterone levels. J Reprod Fertil 46:1-6.

477 Lenko HL, Lang U, Aubert ML, Paunier L, Sizonenko PC (1981) Melatonin in plasma and urine before and during puberty. Pediatr Res 15:74 (abstract).

478 Leonard JM, Leach RB, Couture M, Paulsen CA (1972) Plasma and urinary follicle-stimulating hormone levels in oligospermia. J Clin Endocrinol Metab 34:209-214.

479 Lewis UJ, Singh RNP, Seavy BK (1971) Human prolactin: isolation and some properties. Biochem Biophys Res Commun 44:1169-1176.

480 Leydig F (1850) Zur Anatomie der mämlicken Geschlechtsorgane und Analdrüsen der Säugethiere. Z Wiss Zool 2:1-57.

481 L'Hermite M, Midgeley Jr AR (1971) Radioimmunoassay of human follicle stimulating hormone with antisera to the ovine hormone. J Clin Endocrinol Metab 33: 68-76.

482 Li CH, Starman B (1964) Molecular weight of sheep pituitary interstitial cell stimulating hormone. Nature 202:291-292.

483 Liao S (1975) Cellular receptors and mechanisms of action of steroid hormones. Int Rev Cytol 41:87-172.

484 Liao S, Leninger KR, Sagher D, Barton RW (1965) Rapid effect of testosterone on ribonucleic acid polymerase activity of rat ventral prostate. Endocrinology 77:763-765.

485 Liao S, Liang T, Fang S, Castaneda E, Shao FC (1973a) Steroid structure and androgenic activity. Specificities involved in the receptor binding and nuclear retention of various androgens. J Biol Chem 248:6154-6162.

486 Liao S, Liang T, Tymoczko JL (1973b) Ribonucleo-protein binding of steroid "receptor" complexes. Nature (New Biol) 241:211-213.

487 Liao T-H, Pierce JG (1970) The presence of a common type of subunit in bovine thyroid stimulating and luteinizing hormone. J Biol Chem 245:3275-3281.

488 Libbus BL, Scheutz AW (1979) Reinitiation of spermatogenesis by hormones as assessed by cellular parameters in vitro. Biol Reprod 21:353-364.

489 Libbus BL, Scheutz AW (1980) Gonadotrophin-induced reinitiation of meiosis in testes of oestradiol-treated prepubertal rats. J Reprod Fertil 60:1-6.

490 Licoln GA (1978) The temporal relationship between plasma levels of FSH and LH in the ram. J Reprod Fertil 53:31-37.

491 Licoln GA (1979) Differential control of luteinizing hormone and follicle-stimulating hormone by luteinizing hormone releasing hormone in the ram. J Endocrinol 80:133-140.

492 Licoln GA (1981) Seasonal aspects of testicular function. In: The testis. Eds: H Burger, DM de Kretser. Raven Press, New York, pp255-302.

493 Licoln GA, Rowe PH, Racey RA (1974) The circadian rhythm in plasma testosterone concentration in man. In: Chronological aspects of endocrinology. Eds: J Aschoff, F Ceresa, F Halberg. Schattauer Verlag GmbH, Stuttgart, pp137-149.

494 Licoln GA, Short RV (1980) Seasonal breeding: nature's contraceptive. Recent Prog Horm Res 36: 1-52.

495 Lieberman S (1967) Steroid sulfate as biosynthetic intermediates. Excerpta Med Int Congr Series 132: 22-36.

496 Linde R, Doelle GC, Alexander N, Kirchner F, Vale WW, Rivier J, Rabin D (1981) Reversible inhibition of testicular steroidogenesis and spermatogenesis by a potent gonadotropin-releasing hormone agonist in normal men. N Engl J Med 305:663-667.

497 Lipschultz LI, Caminos-Torres R, Greenspan CS, Snyder PJ (1976) Testicular function after orchidopexy for unilaterally undescended testis. N Engl J Med 295:15-18.

498 Lipschultz LI, Greenberg SH, Caminos-Torres R, Snyder PJ (1977) Supranormal FSH response to gonadotrophin-releasing hormone in oligospermic men with a normal basal serum FSH concentration. Clin Endocrinol 7:103-109.

499 Lipsett MB (1970) Steroid secretion by the human testis. Adv Exp Med Biol 10:407-418.

500 Lipsett MB, Wilson H, Kirschner MA, Korenman LM, Fishman GA, Sarafaty GA, Bardin CW (1966) Studies on Leydig cell physiology and pathology: secretion and metabolism of testosterone. Recent Prog Horm Res 22:245-281.

501 Lloyd HM, Meares JD, Jacobi J (1975) Effects of oestrogens and bromocriptine on in vivo secretion and mitosis in prolactin cells. Nature 255:497-498.

502 Longcope C (1972) The metabolism of estrone sulphate in normal males. J Clin Endocrinol Metab 34:113-122.

503 Longcope C, Kato T, Horton R (1969) Conversion of blood androgens to estrogens in normal adult men and women. J Clin Invest 48:2191-2201.

504 Longcope C, Layne DS, Tait JF (1968) Metabolic clearance rates and interconversions of estrone and 17β-estradiol in normal males and females. J Clin Invest 47:93-106.

505 Longcope C, Widrich W, Sawain CT (1972) The secretion of oestrone and oestradiol-17β by human testis. Steroids 20:439-446.

** 506 Loriaux DL, Ruder HJ, Lipsett MB (1971) The measurement of oestrone sulphate in plasma. Steroids 18: 463-472.

507 Lostroh AJ (1963) Effect of follicle-stimulating hormone and interstitial cell-stimulating hormone on spermatogenesis in Long-Evans rats hypophysectomized for six months. Acta Endocrinol 43:592-600.

508 Lostroh AJ (1969) Regulation by FSH and ICSH (LH) of reproductive function in the immature rat. Endocrinology 85:438-445.

509 Louis BG, Fritz IB (1977) Stimulation by androgens of the production of androgen binding protein by cultured Sertoli cells. Mol Cell Endocrinol 7:9-16.

510 Lucky AW, Rich BH, Rosenfeld RL, Fang VS, Rochebender N (1980) LH bioactivity increases more than immunoreactivity during puberty. J Paediatr 97:205-213.

511 Ludwig DJ (1950) The effect of androgen on spermatogenesis. Endocrinology 46:453-481.

512 Lugaro G, Castellato MM, Mazzola G, Faduni G, Carrea G (1974) Evidence for the existence in spermatozoa of a factor inhibiting the follicle stimulating hormone releasing hormone synthesis. Neuroendocrinology 15:62-68.

513 Lyon M, Glemister P, Lamoreaux M (1975) Normal spermatozoa from androgen-resistant germ cells of chimaeric mice and the role of androgen in spermatogenesis. Nature 258:620-622.

514 Lyon MF, Hawkes SG (1970) X-linked gene for testicular feminization in the mouse. Nature 227: 1217-1219.

515 McCann SM (1962) A hypothalamic luteinizing hormone releasing factor. Am J Physiol 202:395-400.

** Longcope C, Tait JF (1971) see Ref. No. 976

516 McCann SM (1980) Control of anterior pituitary hormone release by brain peptides. Neuroendocrinology 31:355-363.

517 McCann SM, Ojeda SR (1976) Synaptic transmitters involved in the release of hypothalamic releasing and inhibiting hormones. In: Reviews of Neuroscience Vol 2. Eds: S Ehrenpreis, IJ Kopin. Raven Press, New York, pp91-110.

518 McCann SM, Raimirez VD (1964) The neuroendocrine regulation of hypophyseal luteinizing hormone. Recent Prog Horm Res 20:131-170.

519 McCann SM, Taleisnik S, Friedman HM (1960) LH-releasing activity in hypothalamic extracts. Proc Soc Exp Biol Med 104:432-434.

520 McCullagh DR (1932) Dual endocrine activity of testes. Science 76:19-20.

521 McCullagh DR, Walsh EL (1935) Experimental hypertrophy and atrophy of the prostate gland. Endocrinology 19:466.

522 McDonald P, Beyer C, Newton F, Brien B, Baker R, Tan HS, Sampson C, Kitching P, Greenhill R, Pritchard D (1970) Failure of 5α-dihydrotestosterone to initiate sexual behaviour in the castrated male rat. Nature 227:964-965.

523 McEwen BS (1980) Gonadal steroids: humoral modulators of nerve-cell function. Mol Cell Endocrinol 18:151-164.

524 McGill Jr HC, Anselmo VC, Buchanan JM, Sheridan PJ (1980) The heart is a target organ for androgen. Science 207:775-777.

525 McIlree M, Price W, Court Brown WM, Selby Tulloch W, Newsam JE, MacLean N (1966) Chromosome studies on testicular cells from 50 subfertile men. Lancet ii: 69-71.

526 Macleod J, Gold RZ (1951) The male factor in fertility and infertility II. Spermatozoan counts in 1000 men of known fertility and in 1000 cases of infertile marriage. J Urol 66:436-449.

527 McNatty KP, Baird DT, Bolton A, Chambers P, Corker CS, McLean H (1976) Concentration of oestrogen and androgens in human ovarian venous plasma and follicular fluid throughout the menstrual cycle. J Endocrinol 71:77-85.

528 McNeilly AS, de Kretser DM, Sharpe RM (1979) Modulation of prolactin, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) secretion by LHRH and bromocriptine (CB154) in the hypophysectomized pituitary grafted male rat and its effect on testicular LH receptors and testosterone output. Biol Reprod 21:141-147.

529 McNeilly AS, Hagen C (1974) Prolactin, TSH, LH and FSH responses to a combined LHRH/TRH test at different stages of the menstrual cycle. Clin Endocrinol 3:427-435.

530 Mahoudeau JA, Bardin CW, Lipsett MB (1971) The metabolic clearance rate and origin of plasma dihydrotestosterone in man and its conversion to 5α androstanediols. J Clin Invest 50:1338-1344.

531 Main SJ, Davies RV, Setchell BP (1978) Feedback control by the testis of gonadotrophin secretion: an examination of the inhibin hypothesis. J Endocrinol 79:255-270.

532 Main SJ, Davies RV, Setchell BP (1979) The evidence that inhibin must exist. J Reprod Fertil 26:3-14.

533 Main SJ, Davies RV, Setchell BP (1980) Effects of passive immunization against testosterone in serum and pituitary gonadotrophins: implications for the relative importance of androgens and inhibin in the control of gonadotrophin secretion in the male rat. J Endocrinol 86:135-146.

534 Main SJ, Setchell BP (1980) Responsiveness of the pituitary gland to androgens and of the testis to gonadotrophins following damage to spermatogenesis in rats. J Endocrinol 87:445-454.

535 Mainwaring WIP (1969) A soluble androgen receptor in the cytoplasm of rat prostate. J Endocrinol 45: 531-541.

536 Mainwaring WIP (1977) Modes of action of anti-androgens: A survey. In: Androgens and Antiandrogens Eds: L Martini, M Motta. Raven Press, New York, pp151-162.

537 Mainwaring WIP (1980) The Androgens. In: Reproduction in Mammals Book 7: Mechanism of Hormone Action. Eds: CA Austin, RV Short. Cambridge University Press, Cambridge, pp117-156.

538 Mainwaring WIP, Irving RA (1973) The use of deoxyribonucleic acid - cellulose chromatography and isoelectric focusing for the characterization and partial purification of steroid-receptor complexes. Biochem J 134:113-127.

539 Mainwaring WIP, Mangan FR, Feherty PA, Friefeld M (1974) An investigation into the antiandrogenic properties of the non steroidal compound, SCH 13521 (4'-nitro-3'-trifluromethylisobutyrlanilide). Mol Cell Endocrinol 1:113-128.

540 Mainwaring WIP, Peterkin BM (1971) A reconstituted cell-free system for the specific transfer of steroid-receptor complexes into nuclear chromatin isolated from rat ventral prostate gland. Biochem J 125:285-295.

541 Mancini RE, Narbaitz R, Lavieri JC (1960) Origin and development of the germinative epithelium and Sertoli cells in the human testis: cytological, cytochemical and quantitative study. Anat Rec 136:477-489.

542 Mancini RE, Vilar O, Donini P, Perez Lloret A (1971) Effect of human urinary FSH and LH on the recovery of spermatogenesis in hypophysectomized patients. J Clin Endocrinol Metab 33:888-895.

543 Mann T (1975) Biochemistry of semen. In: Handbook of Physiology Section 7 Vol V. Eds: RO Greep, EB Astwood. American Physiological Society, Washington DC, pp461-471.

544 Marian J, Conn PM (1979) Gonadotropin releasing hormone stimulation of pituitary cells requires calcium. Mol Pharmacol 16:196-201.

545 Markee JE, Everett JW, Sawyer CH (1952) The relationship of the nervous system to the release of gonadotrophin and the regulation of the sex cycle. Recent Prog Horm Res 7:139-164.

546 Markee JE, Sawyer CH, Hollinshead WH (1946) Activation of the anterior hypophysis by electrical stimulation in the rabbit. Endocrinology 38:345-357.

547 Marriot FHC (1974) The interpretation of multiple observations. Academic Press, New York, pp32-43.

548 Marshall FHA (1904) The oestrous cycle in the common ferret. Q J Microsc Sci 48:323-345.

549 Marshall FHA (1936) Sexual periodicity and the causes which determine it. The Croonian Lecture. Philos Trans R Soc Lond (Biol) 226:423-456.

550 Marshall FHA (1942) Exteroceptive factors in sexual periodicity. Biol Rev 17:68-90.

551 Marshall FHA, Verney EB (1936) The occurrence of ovulation and pseudopregnancy in the rabbit as a result of central nervous system stimulation. J Physiol 86:327-336.

552 Marshall JC, Anderson DC, Fraser TR, Harsoulis P (1973) Human luteinizing hormone in man. Studies of metabolism and biological action. J Endocrinol 56: 431-439.

553 Marshall WA (1974) Interrelationship of skeletal maturation, sexual development and somatic growth in man. Ann Hum Biol 1:29-40.

554 Marshall WA, Tanner JM (1969) Variations in pattern of pubertal changes in girls. Arch Dis Child 44: 291-303.

555 Marshall WA, Tanner JM (1970) Variations in the pattern of pubertal changes in boys. Arch Dis Child 45:13-23.

556 Marshall WA, Tanner JM (1974) Puberty. In: Scientific Foundations of Paediatrics. Eds: JA Davies, J Dobbing. Heinemann, London, pp124-151.

557 Martensz ND, Scaramuzzi RJ, Van Look PFA (1979) Plasma concentrations of luteinizing hormone and follicle-stimulating hormone during anoestrous in ewes actively immunized against oestradiol-17β, oestrone or testosterone. J Endocrinol 81:261-269.

558 Martins T, Rocha A (1931) Regulation of the hypophysis by the testis and some problems of sexual dynamics. Endocrinology 15:421-434.

559 Matsumoto A, Arai Y (1977) Precocious puberty and synaptogenesis in the hypothalamic arcuate nucleus in pregnant mare serum gonadotrophin (PMSG) treated immature female rats. Brain Res 129:375-378.

560 Matsuo H, Arimura A, Nair RMG, Schally AV (1971b) Synthesis of the porcine LH- and FSH-releasing hormone by the solid-phase method. Biochem Biophys Res Commun 45:822-827.

561 Matsuo H, Baba Y, Nair RMG, Arimura A, Schally AV (1971a) Structure of the porcine LH- and FSH-releasing hormone I. The proposed amino acid sequence. Biochem Biophys Res Commun 43:1334-1339.

562 Mauss J, Börsch G (1973) Immunochemical assay of follicle-stimulating and luteinizing hormone in the unconcentrated urine of sub- or infertile males. Acta Endocrinol 74:631-641.

563 Means AR, Dedman JR (1980) Calmodulin: an intracellular calcium receptor. Nature 285:73-76.

564 Means AR, Dedman JR, Tash JS, Tindall DJ, van Sickle M, Welsh MJ (1980) Regulation of the testis Sertoli cell by follicle-stimulating hormone. Annu Rev Physiol 42:59-70.

565 Means AR, Dedman JR, Tindall DJ, Welsh MJ (1978) Hormonal regulation of Sertoli cells. Int J Androl (Suppl) 2:403-421.

566 Means AR, Fakunding JL, Huckins C, Tindall DJ, Vitale R (1976) Follicle-stimulating hormone, the Sertoli cell and spermatogenesis. Recent Prog Horm Res 32:477-527.

567 Mecklenburg RS, Sherins RJ (1974) Gonadotropin response to luteinizing hormone-releasing hormone in men with germinal aplasia. J Clin Endocrinol Metab 38:1005-1008.

568 Medeiros-Neto GA, Toledo SPA, Pupo AA, Sucupira MS, Fraige-Filho F, Mattar E, Kastin AJ, Schally AV (1973) Characterization of the LH response to luteinizing hormone-releasing hormone (LH-RH) in isolated and multiple tropic hormone deficiencies. J Clin Endocrinol Metab 37:972-976.

569 Meinhard E, McRae CU, Chisholm GD (1973) Testicular biopsy in evaluation of male infertility. Br Med J 3:577-581.

570 Meites J, Bruni JF, Van Vugt DA, Smith AF (1979) Relation of endogenous opioid peptides and morphine to neuroendocrine functions. Life Sci 24:1325-1336.

571 Melicow MM, Pulrang S (1974) Castrati choir and opera singers. Urology 3:663-670.

572 Mendelson C, Dufau ML, Catt KJ (1975) Gonadotrophin binding and stimulation of cyclic adenosine 3'5' monophosphate and testosterone production in isolated Leydig cells. J Biol Chem 250:8818-8823.

573 Menon KMJ, Dorfman RI, Forchielli E (1967) Influence of gonadotrophins on the cholesterol side-chain cleavage reaction by rat testis mitochondrial preparations. Biochem Biophys Acta 148:486-494.

574 Mercier-Bodard C, Alfsen A, Baulieu E-E (1966) A testosterone binding globulin. In: Proceedings of the Second Symposium on Steroid Hormones, Ghent 1965. Excerpta Medica International Congress Series 101: 212.

575 Mercier-Bodard C, Renoir J-M, Baulieu E-E (1979) Further characterization and immunological studies of human sex steroid binding plasma protein. J Steroid Biochem 11:253-259.

576 Midgley Jr AR, Jaffe RB (1971) Regulation of human gonadotropins: X. Episodic fluctuation of LH during the menstrual cycle. J Clin Endocrinol Metab 33: 962-969.

577 Migeon CJ, Rivarola MA, Forest MG (1968) Studies of androgens in transexual subjects. Effects of oestrogen therapy. Bull Johns Hopkins Hosp 123: 128-133.

578 Mills NC, Mills TM, Yurkiewicz WJ, Bardin CW (1979) Actions of androgens on the kidney of female mice: strain differences in the DNA and β glucuronidase responses. Int J Androl 2:371-384.

579 Miyachi Y, Mecklenberg RS, Hansen JW, Lipsett MB (1973) Metabolism of ^{125}I-luteinizing hormone-releasing hormone. J Clin Endocrinol Metab 37:63-67.

580 Mizutani S, Tsukimura T, Akashi S, Matsumoto K (1977) Lack of metabolism of progesterone, testosterone and pregnenolone to 5α-products in monkey and human testes compared with rodent testes. J Clin Endocrinol Metab 44:1023-1031.

581 Moger WH (1976) Effects of testosterone implants in serum gonadotrophin concentrations in the male rat. Biol Reprod 14:665-669.

582 Moger WH (1977) Serum 5-alpha-androstane-3-alpha, 17 beta-diol, androsterone and testosterone concentrations in male rat - influence of age and gonadotropin stimulation. Endocrinology 100:1027-1032.

583 Moger WH (1979) Production of testosterone, 5α-androstane-3α, 17β-diol and androsterone by dispersed testicular interstitial cells and whole testes in vitro. J Endocrinol 80:321-332.

584 Moger WH (1980) Direct effects of oestrogens on the endocrine function of the mammalian testis. Can J Physiol Pharmacol 58:1011-1022.

585 Moneti G, Pazzagli M, Fiorelli G, Serio M (1980) Measurement of 5α androsterone-3α-17β diol in human spermatic venous plasma by mass fragmentography. J Steroid Biochem 13:623-627.

586 Monet-Kuntz C, Terqui M, Locatelli A, Hochereau-de-Reviers MT, Courot M (1976) Effets de la supplementation en testosterone sur la spermatogenese de belier hypophysectomisés. C R Seances Acad Paris D283:1763-1766.

587 Moor BC, Younglai EV (1975) Variations in peripheral levels of LH and testosterone in adult male rabbits. J Reprod Fertil 42:259-266.

588 Moore CR, Gallagher TF (1930) Seminal vesicle and prostate function as a testis hormone indicator: the electric ejaculation test. Am J Anat 45:39-69.

589 Moore CR, McGee LC (1928) On the effects of injecting lipid extracts of bull testes into castrated guinea pigs. Am J Physiol 87:436-446.

590 Moore CR, Price D (1932) Gonad hormone functions and the reciprocal influence between gonads and hypophysis, with its bearing upon sex hormone antagonism. Am J Anat 50:13-71.

591 Morley JE, Baranetsky NG, Wingert TD, Carlson HE, Hershman JM, Melmed S, Levin SR, Jamison KR, Weitzman R, Chang RJ, Varner AA (1980) Endocrine effects of naloxone-induced opiate receptor blockade. J Clin Endocrinol Metab 50:251-257.

592 Morris ID, Jackson CM (1978) Gonadotrophin response after castration and selective destruction of the testicular interstitium in the normal and aspermatogenic rat. Acta Endocrinol 88:38-47.

593 Morris JM (1953) The syndrome of testicular feminization in male pseudohermaphrodites. Am J Obstet Gynecol 65:1192-1211.

594 Morris JM, Mahesh VB (1963) Further observations on the syndrome "testicular feminization". AM J Obstet Gynecol 87:731-748.

595 Mortimer CH, Besser GM, McNeilly AS (1975) Gonadotrophin releasing hormone therapy in the induction of puberty, potency, spermatogenesis and ovulation in patients with hypothalamic pituitary-gonadal dysfunction. In: Hypothalamic Hormones. Eds: M Motta, PG Crosglinani, L Martini. Academic Press, London, pp325-336.

596 Mortimer CH, McNeilly AS, Fisher RA, Murray MAF, Besser GM (1974) Gonadotrophin-releasing hormone therapy in hypogonadal males with hypothalamic or pituitary dysfunction. Br Med J 4:617-621.

597 Mortimer CH, McNeilly AS, Rees LH, Lowry PJ, Gilmore D, Dobbie HG (1976) Radioimmunoassay and chromatographic similarity of circulating endogenous gonadotropin releasing hormone and hypothalamic extracts in man. J Clin Endocrinol Metab 43:882-888.

598 Moult PJA, Grossman A, Evans JM, Rees LH, Besser GM (1981) The effect of naloxone on pulsatile gonadotropin release in normal subjects. Clin Endocrinol 14:321-324.

599 Mowszowicz I, Kahn D, Dray F (1970) Influence of testosterone binding to serum proteins or aromatization by enzymes of human placental microsomes. J Clin Endocrinol Metab 584-586.

600 Moyle WR, Jungas RL, Greep RO (1973) Metabolism of free and esterified cholesterol by Leydig cell tumour mitochondria. Biochem J 134:415-424.

601 Mulder E, Van Beurden-Lamers WMO, Brinkman AO, Mechielschen MJ, van der Molen HJ (1974) High affinity binding of oestradiol by rat testis interstitial tissue and by several other tissues of the male rat. J Steroid Biochem 5:955-959.

602 Murono EP, Payne AH (1979) Testicular maturation in the rat. In vivo effect of gonadotropins of steroidogenic enzymes in the hypophysectomized immature rat. Biol Reprod 20:911-917.

603 Murray MAF, Corker CS (1973) Levels of testosterone and luteinizing hormone in plasma samples taken at 10-minute intervals in normal men. J Endocrinol 56: 157-158.

604 Murty GSRC, Sheela Rani CS, Moudgal NR, Prasad MRN (1979) Effect of passive immunization with specific antiserum to FSH on the spermatogenic process and fertility of adult male Bonnet monkeys (Macaca radiata). J Reprod Fertil (Suppl) 26:147-163.

605 Musto N, Hafiez AA, Bartke A (1972) Prolactin increases 17β-hydroxysteroid dehydrogenase activity in the testis. Endocrinology 91:1106-1108.

606 Naess O, Haug E, Attramadal A, Aakvaag A, Hansson V, French F (1976) Androgen receptors in the anterior pituitary and central nervous system of the androgen "insensitive" (Tfm) rat. Endocrinology 99:1295-1303.

607 Naftolin F, Judd HL, Yen SSC (1973) Pulsatile patterns of gonadotrophins and testosterone in man: the effects of clomiphene with and without testosterone. J Clin Endocrinol Metab 36:285-288.

608 Naftolin F, Ryan KJ, Davies IJ, Reddy VV, Flores F, Petro Z, Kuhn M, White RJ, Wolin L, Takaoka Y (1975) The formation of estrogens by central neuroendocrine tissues. Recent Prog Horm Res 31:295-320.

609 Nankin HR, Castaneda E, Troen P (1977) Endocrine profiles in oligospermic men. In: The testis in normal and infertile men. Eds: HR Nankin, P Troen. Raven Press, New York, pp529-537.

610 Nankin HR, Troen P (1971) Repetitive luteinizing hormone elevations in serum of normal men. J Clin Endocrinol Metab 33:558-560.

611 Nankin HR, Troen P (1972) Overnight patterns of serum luteinizing hormone in normal men. J Clin Endocrinol Metab 35:705-710.

612 Naor Z, Catt KJ (1980) Independent actions of GnRH upon cyclic GMP production and LH release. J Biol Chem 255:342-344.

613 Naor Z, Fawcett CP, McCann SM (1978) Involvement of cGMP in LHRH stimulated gonadotropin release. Am J Physiol 235:E586.

614 Nayfeh SN, Barefoot SW, Baggett B (1966) Metabolism of progesterone by rat testicular homogenates II. Changes with age. Endocrinology 78:1041-1048.

615 Nayfeh SN, Coffey JC, Hansson V, French FS (1975) Maturation changes in testicular steroidogenesis: Hormonal regulation of 5α reductase. J Steroid Biochem 6:329-335.

616 Negro-Vilar A, Krulich L, McCann SM (1973) Changes in serum prolactin and gonadotropins during sexual development of the male rat. Endocrinology 93: 660-664.

617 Neil JD (1974) Prolactin: its secretion and control. In: Handbook of Physiology Section 7, Endocrinology. Eds: E Knobil, WH Sawyer; Vol IV, Part 2. American Physiological Society, Washington DC, pp469-488.

618 Neil JD, Patton JM, Dailey RA, Tsou RC, Tindall GT (1977) Luteinizing hormone releasing hormone (LHRH) in pituitary stalk blood of rhesus monkeys: relationship to level of LH release. Endocrinology 101: 430-434.

619 Nelson WO (1953) Interpretation of testicular biopsy. J A M A 151:449-454.

620 Nelson WO, Merckel CE (1938) Maintenance of spermatogenesis in hypophysectomized mice with androgenic substances. Proc Soc Exp Biol Med 38:737-740.

621 Nett TM, Akbar AM, Nieswender GD, Hedlund MT, White WF (1973) A radioimmunoassay for gonadotrophin-releasing hormone GnRH in serum. J Clin Endocrinol Metab 36:880-885.

622 Nett TM, Crowder ME, Moss GE, Duello TM (1981) GnRH-receptor interaction V. Down regulation of pituitary receptors for GnRH in ovariectomized ewes by infusion of homologous hormone. Biol Reprod 24:1145-1155.

623 Nicoll CS, Buntin J, Clemons G, Schreibman M, Russell S (1977) Comparative aspects of prolactin physiology: radioimmunoassay of the hormone in nonmammalian vertebrates. In: Endocrinology Vol 2. Ed: VHT James. Excerpta Medica, Amsterdam, pp293-297.

624 Nieschlag E (1974) Circadian rhythm of plasma testosterone. In: Chronobiological Aspects of Endocrinology. Eds: Aschoff J, Ceresa F, Halberg F. Schattauer Verlag GmbH, Stuttgart, pp117-128.

625 Nieschlag E, Usadel KH, Kley HK (1975) Active immunization with steroids as an approach to investigating testicular and adrenal feedback control. J Steroid Biochem 6:537-540.

626 Nieschlag E, Wickings EJ, Mauss J (1978) Endocrine testicular function in infertility. In: Recent Progress in Andrology: Proceedings of Serono Symposium Vol 14. Eds: A Fabbrini, E Steinberger. Academic Press, London, pp101-113.

627 Nieschlag E, Wickings EJ, Mauss J (1979) Endocrine testicular function in vivo and in vitro in infertile men. Acta Endocrinol 90:544-551.

628 Nokin J, Vekemans M, L'Hermite M, Robyn C (1972) Circadian periodicity of serum prolactin concentration in man. Br Med J 3:561-562.

629 Odell WD, Swerdloff RS (1976) Etiologies of sexual maturation: a model system based on the sexually maturing rat. Recent Prog Horm Res 32:245-288.

630 Odell WD, Swerdloff RS, Bain J, Wollensen F, Grover PK (1974) The effect of sexual maturation on testicular response to LH stimulation of testosterone secretion in the intact rat. Endocrinology 95: 1380-1384.

631 Oertel GW, Eik-Nes KB (1962) Two pathways for the formation of testosterone in vivo. In: Gewebs and Neurohormone, Ed: H Nowakoski. Springer-Verlag, New York, pp340.

632 Oh R, Tamaoki B (1973) Intermicrosomal distribution of aromatizing enzyme system in equine testicular tissue. Acta Endocrinol 72:366-375.

633 Ohno S (1976) Major regulatory genes for mammalian sexual development. Cell 7:315-321.

634 Ojeda SR, Advis JP, Andrews AA (1980) Neuroendocrine control of the onset of puberty in the rat. Fed Proc 39:2365-2371.

635 Ojeda SR, McCann SM (1978) Control of LH and FSH release by LHRH: influence of putative neurotransmitters. Clin Obstet Gynecol 5(2):281-303.

636 Ojeda SR, Negro-Vilar A, McCann SM (1979) Release of prostaglandin Es by hypothalamic tissue: evidence for their involvement in catecholamine-induced luteinizing hormone-releasing hormone release. Endocrinology 104:617-624.

637 Olivo J, Southren AL, Gordon GG, Tochimoto S (1970) Studies of the protein binding of testosterone in plasma in disorders of thyroid function: effect of therapy. J Clin Endocrinol Metab 31:539-545.

638 Orth J, Christensen AK (1977) Localization of ^{125}I-labelled FSH in the testes of hypophysectomized rats by autoradiography at the light and electromicroscope levels. Endocrinology 101:262-278.

639 Oshima H, Nankin HR, Troen P, Yoshida KI, Ochinai KI (1977) Leydig cell number and function in infertile men. In: The testis in normal and infertile men. Eds: P Troen, HR Nankin. Raven Press, New York, pp445-465.

640 Padron RS, Wischuen J, Hudson B, Burger HG, de Kretser DM (1980) Prolonged biphasic response of plasma testosterone to single intramuscular injections of human chorionic gonadotrophin. J Clin Endocrinol Metab 50:1100-1104.

641 Pahnke VG, Leidenberger FA, Kunzig HJ (1975) Correlation between hCG (LH)-binding capacity, Leydig cell number and secretory activity of rat testis throughout pubescence. Acta Endocrinol 79:610-618.

642 Paigen K, Swank RT, Tomino S, Gamshow RE (1975) The molecular genetics of mammalian glucuronidase. J Cell Physiol 85:379-392.

643 Parker DC, Judd HL, Rossman LG, Yen SSC (1975) Pubertal sleep-wake patterns of episodic LH, FSH and testosterone release in twin boys. J Clin Endocrinol Metab 40:1099-1109.

644 Parker DC, Rossma LG, Vander Laan EF (1973) Sleep related nyctohemeral and briefly episodic variation in human prolactin concentration. J Clin Endocrinol Metab 36:1119-1124.

645 Parker DC, Sassin JF, Mace JW, Gotlin RW, Rossman LG (1969) Human growth hormone release in sleep: electroencephalographic correlation. J Clin Endocrinol Metab 29:871-874.

646 Parker LN, Sach J, Fisher DA, Odell WD (1978) The adrenarche in prolactin, gonadotrophins, adrenal androgens and cortisol. J Clin Endocrinol Metab 46: 396-401.

647 Pavel S (1979) The mechanism of action of vasotocin in the mammalian brain. Prog Brain Res 52:445-458.

648 Pavel S, Petrescu S (1966) Inhibition of gonadotrophin by a highly purified pineal peptide and by synthetic arginine vasotocin. Nature 212:1054.

649 Payne AH, Downing JR, Wong K-L (1980a) Luteinizing hormone receptors and testosterone synthesis in two distinct populations of Leydig cells. Endocrinology 106:1424-1430.

650 Payne AH, Jaffe RB (1975) Androgen formation from pregnenolone sulfate by fetal, neonatal, prepubertal and adult human testes. J Clin Endocrinol Metab 40: 102-107.

651 Payne AH, Jaffe RB, Abell MR (1971) Gonadal steroid sulfates and sulfatase III. Correlation of human testicular sulfatase, 3β-hydroxysteroid dehydrogenase-isomerase, histologic structure and serum testosterone. J Clin Endocrinol Metab 33:582-591.

652 Payne AH, Kawano A, Jaffe RB (1973) Formation of dihydrotestosterone and other 5α-reduced metabolites by isolated seminiferous tubules and suspension of interstitial cells in a human testis. J Clin Endocrinol Metab 37:448-453.

653 Payne AH, Kelch RP, Murono EP, Kerlan JT (1977) Hypothalamic, pituitary and testicular function during sexual maturation of the male rat. J Endocrinol 72:17-26.

654 Payne AH, Kelch RP, Musich SS, Halpern ME (1976) Intratesticular site of aromatization in the human. J Clin Endocrinol Metab 42:1081-1087.

655 Payne AH, Wong K-L, Vega MM (1980b) Differential effects of single and repeated administrations of gonadotropins on luteinizing hormone receptors and testosterone synthesis in two populations of Leydig cells. J Biol Chem 255:7118-7122.

656 Pazzagli M, Borrelli E, Forti G, Serio M (1974) Dihydrotestosterone in human spermatic venous plasma. Acta Endocrinol 76:388-391.

657 Pearlman WH, Crépy O, Murphy M (1967) Testosterone-binding levels in the serum of women during the normal menstrual cycle, pregnancy and the post-partum period. J Clin Endocrinol Metab 27:1012-1018.

658 Peillon F, Bard H, Mowszowicz I, Cesselin F, Lagoguey M, Boyet F (1979) Prolactin-secreting adenomas in males. Study of the hypophyseal gonadal axis. Ann Endocrinol (Paris) 40:73-74.

659 Perloff WH (1949) Role of hormones in human sexuality. Psychosom Med 11:133-139.

660 Peterson RE, Imperato-McGinley J, Gautier T, Sturla E (1977) Male pseudohermaphroditism due to steroid 5α reductase deficiency. Am J Med 62:170-190.

661 Pirke KM, Sintermann R, Doerr P (1977) Testosterone and testosterone precursors in the spermatic vein of young and old men. Acta Endocrinol (Suppl) 84:78-79.

662 Plant TM (1981) Time courses of concentrations of circulating gonadotropin, prolactin, testosterone and cortisol in adult male Rhesus monkeys (Macaca mulatta) throughout the 24h light-dark cycle. Biol Reprod 25:244-252.

663 Plant TM, Hess DL, Hotchkiss J, Knobil E (1978) Testosterone and the control of gonadotrophin secretion in the male rhesus monkey. Endocrinology 103:535-541.

664 Plant TM, Schallenberger E, Hess DL, McCormack JT, Dufy-Barbe L, Knobil E (1980) The influence of suckling on gonadotropin secretion in the female rhesus monkey (Macaca mulatta). Biol Reprod 23: 760-766.

665 Pomerantz DK (1979) Effects of in vivo gonadotropin treatment on estrogen levels in the testis of the immature rat. Biol Reprod 21:1247-1255.

666 Popa GT, Fielding U (1930) A portal circulation from the pituitary to the hypothalamic region. J Anat 65:88-91.

667 Popa GT, Fielding U (1933) Hypophysio-portal vessels and their colloid accompaniment. J Anat 67:227-232.

668 Prader A (1966) Testicular size: assessment and clinical importance. Triangle 7:240-243.

669 Prader A (1975) Delayed adolescence. Clin Endocrinol Metab 4 (1):143-154.

670 Price D, Ortiz E (1944) The relation of age to reactivity in the reproductive system of the rat. Endocrinology 34:215-239.

671 Pritchard JB (ed)(1955) Ancient Near Eastern Texts 2nd edition. Princeton University Press, Princeton, New Jersey.

672 Pryor JP, Cameron KM, Collins WP, Hirsh AV, Mahony JDH, Pugh RCB, Fitzpatrick JM (1978) Indications for testicular biopsy or exploration in azoospermia. Br J Urol 50:591-594.

673 Pryor JP, Pugh RCB, Cameron KM, Newton JR, Collins WP (1976) Plasma gonadotrophic hormones, testicular biopsy and seminal analysis in the men of infertile marriages. Br J Urol 48:709-717.

674 Purdy RH, Engel LL, Oncley JL (1961) The characterization of estrone sulphate from human plasma. J Biol Chem 236:1043-1050.

675 Purvis K, Brenner PF, Landgren B-M, Cekan Z, Diczfalusy E (1975) Indices of gonadal function in the human male I. Plasma levels of unconjugated steroids and gonadotrophins under normal and pathological conditions. Clin Endocrinol 4:237-246.

676 Purvis K, Clausen OPF, Hansson V (1978a) Functional characteristics of rat Leydig cells. Ann Biol Anim Biochem Biophys 18:595-605.

677 Purvis K, Clausen OPF, Hansson V (1978b) Decreased Leydig cell responsiveness in the testicular feminized male rat. Endocrinology 102:1053-1060.

678 Purvis K, Clausen OPF, Hansson V (1978c) Regulation of Leydig cell sensitivity and responsiveness to LH/hCG. Int J Androl (Suppl) 2:247-263.

679 Purvis K, Clausen OPF, Hansson V (1980) Effects of age and hypophysectomy on responsiveness of rat Leydig cells to hCG. J Reprod Fertil 60:77-86.

680 Purvis K, Hansson V (1978) Hormonal regulation of Leydig cell function. Mol Cell Endocrinol 12: 123-128.

681 Purvis K, Olsen A, Haug E, Hansson V (1979) Prolactin and Leydig cell responsiveness to LH/hCG in the rat. Arch Androl 3:219-230.

682 Quigley ME, Sheehan KL, Caspar RF, Yen SSC (1980) Evidence for increased dopaminergic and opioid activity in patients with hypothalamic hypogonadotropic amenorrhoea. J Clin Endocrinol Metab 50: 949-954.

683 Quigley ME, Yen SSC (1980) The role of endogenous opiates on LH secretion during the menstrual cycle. J Clin Endocrinol Metab 51:179-181.

684 Raboch J, Starka L (1971) Hormonal testicular activity in men with a varicocoele. Fertil Steril 22:152-155.

685 Raeside JI (1969) The isolation of estrone sulphate and oestradiol sulphate from stallion testis. Can J Biochem 47:811-815.

686 Raimirez VD (1973) Endocrinology of puberty. In: Handbook of Physiology Vol II Part 1. Eds: RO Greep, EB Astwood, ppl-28.

687 Raimirez VD, McCann SM (1963) Comparison of the regulation of luteinizing hormone (LH) secretion in immature and adult rats. Endocrinology 72:452-464.

688 Raiti S, Foley Jr TP, Penny R, Blizzard RM (1975) Measurement of theproduction rate of human luteinizing hormone using the urinary extraction technique. Metabolism 24:937-941.

689 Ramaley JA (1980) Biological clocks and puberty onset. Fed Proc 39:2355-2359.

690 Rebar R, Yen SSC, Vandenberg G, Naftolin F, Ehara Y, Engblom S, Ryan RJ, Rivier J, Amoss M, Guillemin R (1973) Gonadotropin responses to synthetic LRF: dose response relationship in men. J Clin Endocrinol Metab 36:10-16.

691 Rechtschaffen A, Kales A (1968) A manual of standardized terminology, techniques and scoring system for sleep stages of human subjects. U.S. Government Printing Office, Washington DC.

692 Reeves J (1979) Estimating fatness (letter). Science 204:881.

693 Rege N, Phadke A, Bhatt J, Khatri N, Sheth A, Joshi U, Vaidya R (1979) Serum gonadotrophins and testosterone in infertile patients with varicocoele. Fertil Steril 31:413-416.

694 Rehan N-E, Sobrero AJ, Fertig JN (1975) The semen of fertile men: statistical analysis of 1300 men. Fertil Steril 26:492-502.

695 Reinberg A, Lagoney M, Chauffourrier JM, Cesslin F (1975) Circannual and circadian rhythms in plasma testosterone in five healthy young Parisian males. Acta Endocrinol 80:752-743.

696 Reitano JF, Caminos-Torres R, Snyder PJ (1975) Serum LH and FSH responses to the repetitive administration of gonadotropin-releasing hormone in patients with idiopathic hypogonadotrophic hypogonadism. J Clin Endocrinol Metab 41:1035-1042.

697 Reiter EO, Kaplan SL, Conte FA, Grumbach MM (1975) Responsivity of pituitary gonadotropes to luteinizing hormone releasing factor in precocious puberty, precocious thelarche, precocious adrenarch and in patients treated with medroxyprogesterone acetate. Pediatr Res 9:111-116.

698 Reiter EO, Root AW, Duckett GE (1976) The response of pituitary gonadotropes to a constant infusion of LHRH in normal prepubertal and pubertal children and in children with abnormalities of sexual development. J Clin Endocrinol Metab 43:400-411.

699 Reiter RJ (1974) Pineal regulation of hypothalamico-pituitary axis: gonadotrophins. In: Handbook of Physiology, Endocrinology Vol 4 Part 2. Eds: E Knobil, WH Sawyer. American Physiological Society, Washington DC, pp519-550.

700 Reiter RJ (1980) Seasonal aspects of testicular function in male photoperiodic rodents. In: Testicular Development, Structure and Function. Eds: A Steinberger, E Steinberger. Raven Press, New York, pp395-400.

701 Reiter RJ, Vaughan MK (1977) Pineal antigonadotrophic substances: polypeptides and indoles. Life Sciences 21:159-172.

702 Resko AJ, Quadrai SK, Spies HG (1977) Negative feedback control of gonadotrophins in male rhesus monkeys: effects of time after castration and interactions of testosterone and estradiol-17β. Endocrinology 101:215-224.

703 Reynolds EL, Wines JV (1951) Physical changes associated with adolescence in boys. Am J Dis Child 82:529-549.

704 Rich AK, Kerr JB, de Kretser DM (1979) Evidence of Leydig cell dysfunction in rats with seminiferous tubular damage. Mol Cell Endocrinol 13:123-135.

705 Richardson DW, Short RV (1978) Time of onset of sperm production in boys. J Biosoc Sci (Suppl) 5: 15-25.

706 Rifka SM, Sherins RJ (1978) Current concepts in evaluation of the infertile male. Clin Obstet Gynaecol 5(2):481-497.

707 Risbridger GP, Hodgson YM, de Kretser DM (1981) Mechanism of action of gonadotrophin on the testis. In: The Testis. Eds: H Burger, DM de Kretser. Raven Press, New York, pp195-211.

708 Rivarola MA, Podesta EJ, Chemes HE (1972) In vitro testosterone-^{14}C metabolism by rat seminiferous tubules at different stages of development: formation of 5α-androstanediol at meiosis. Endocrinology 91:537-542.

709 Rivarola MA, Saez JM, Meyer WJ, Jenkins ME, Migeon CJ (1966) Metabolic clearance rate and blood production rate of testosterone and androst-4-ene-3, 17-dione under basal conditions, ACTH and HCG stimulation. Comparison with urinary production rate of testosterone. J Clin Endocrinol Metab 26: 1208-1218.

710 Robel P, Lasnitzki I, Baulieu E-E (1971) Hormone metabolism and action: testosterone and metabolites in prostate organ culture. Biochimie 53:81-97.

711 Rodriguez-Rigau LJ, Weiss DB, Smith KD, Steinberger E (1978) Suggestion of abnormal testicular steroidogenesis in some oligospermic men. Acta Endocrinol 87:400-412.

712 Rolland R, Lequin RM, Schellekens LA, de Jong FH (1975) The role of prolactin in the restoration of ovarian function during the early post partum period in the human female I. A study during physiological lactation. Clin Endocrinol 4:15-25.

713 Rommerts FFG, Brinkman AO (1981) Modulation of steroidogenic activities in testis Leydig cells. Mol Cell Endocrinol 21:15-28.

714 Rommerts FFG, Cooke BA, van der Molen HJ (1974) The role of cyclic AMP in the regulation of steroid biosynthesis in the testis tissue. J Steroid Biochem 5:279-285.

715 Rommerts FFG, de Jong FH, Grootgoed JA, van der Molen HJ (1980) Metabolic changes in testicular cells from rats after long-term exposure to 37°C in vivo or in vitro. J Endocrinol 85:471-479.

716 Rommerts FFG, Krüger-Sewnarian BC, Grootgoed JA, de Jong FH, van der Molen HJ (1979) Secretion of protein, androgen binding protein and oestradiol by Sertoli cells in culture: effects of serum. Acta Endocrinol 90:552-561.

717 Rommerts FFG, Krüger-Sewnarian BC, van-Woerkom-Blik A, Grootgoed JA, van der Molen HJ (1978) Secretion of proteins by Sertoli cell enriched cultures: effects of follicle stimulating hormone, dibutryl cAMP and testosterone and correlation with secretion of oestradiol and androgen binding protein. Mol Cell Endocrinol 10:39-55.

718 Root A, de Cherney A, Russ D, Duckett G, Garcia C-R, Wallach E (1972) Episodic secretion of luteinizing and follicle stimulating hormones in agonadal and hypogonadal adolescents and adults. J Clin Endocrinol Metab 35:700-704.

719 Rose LI, Underwood RH, Parker DE, Williams GH (1973) Δ^4-3-ketosteroid 5α-reductase in the adult human testis. J Clin Endocrinol Metab 36:995-998.

720 Rosen SW, Weintraub BD (1971) Monotropic increase of serum FSH correlated with low sperm count in young men with idiopathic oligospermia and aspermia. J Clin Endocrinol Metab 32:410-416.

721 Rosenfeld RL (1969) A competetive protein binding method for the measurement of plasma androstenedione. Steroids 14:251-261.

722 Rosenfeld RL (1971) Plasma testosterone binding globulin and indexes of the concentration of unbound plasma androgens in normal and hirsute subjects. J Clin Endocrinol Metab 32:717-728.

723 Rosenfeld RL, Fang VS, Dupon C, Kim MH, Refetoff S (1973) The effect of low doses of depot estradiol and testosterone in teenagers with ovarian failure and Turner's syndrome. J Clin Endocrinol Metab 37: 574-580.

724 Rosen-Runge EC (1956) Quantitative investigations in human testicular biopsies. Fertil Steril 7:251-261.

725 Rosner W (1972) A simplified method for the quantitative determination of testosterone oestradiol binding globulin activity in human plasma. J Clin Endocrinol Metab 34:983-988.

726 Rosness PA, Eik-Nes KB (1977) Biosynthesis of Androgens. In: Androgens and Antiandrogens. Ed: L Martini, M Motta. Raven Press, New York, ppl-9.

727 Ross GT (1970) Plasma FSH and LH measured by radio-immunoassay in normal and pathological conditions in men. In: The Human Testis. Eds: R Rosemberg, CA Paulsen. Plenum Press, New York, pp289-296.

728 Roth J, Kahn CR, Lesniak MA (1975) Receptors for insulins, NSI-LA-S, and growth hormone: applications to disease states in man. Recent Prog Horm Res 31: 95-249.

729 Roth JC, Kelch RP, Kaplan SL (1972) FSH and LH response to LHRH in prepubertal and pubertal children, adult males and patients with hypogonado-tropic and hypergonadotropic hypogonadism (32 subjects). J Clin Endocrinol Metab 35:926-930.

730 Roth J, Le Roith D, Shiloach J, Rosenweig JL, Lesni k MA, Havrankova J (1982) The evolutionary origins of hormones, neurotransmitters and other extracellular chemical messengers. N Engl J Med 306:523-527.

731 Roulier R, Mattei A, Franchimont p (1976) FSH, LH before and after LHRH in azoospermic and oligo-spermic subjects. Ann Endocrinol (Paris) 37:407-427.

732 Rowe PH, Racey PA, Licoln GA, Ellwood M, Lehane J, Shenton JC (1975) The temporal relationship between secretion of luteinizing hormone and testosterone in man. J Endocrinol 64:17-26.

733 Roy AK (1973) Androgenic induction of alpha-2U globulin in rats: androgen insensitivity in prepubertal animals. Endocrinology 92:957-960.

734 Roy AK, Neuhaus OW (1967) Androgenic control of a sex-dependent protein in the rat. Nature 214: 618-620.

735 Rubin CS, Rosen OM (1975) Protein phosphorylation. Annu Rev Biochem 44:831-887.

736 Rubin RT, Gouin PR, Lubin A, Poland RE, Pirke KM (1975) Nocturnal increase of plasma testosterone in men: relation to gonadotropins and prolactin. J Clin Endocrinol Metab 40:1027-1033.

737 Rubin RT, Kales A, Adler R, Fagan T, Odell W (1972) Gonadotropin secretion during sleep in normal adult men. Science 175:196-198.

738 Rubin RT, Leonard LT, Zir MC, Smith RA, Fagan T, Adler R (1971) Experience with the Vankirk-Sassin technique for serial blood sampling during sleep. Am J EEG Technol 11:17-18.

739 Rubin RT, Poland RE, Touer BB (1976) Prolactin related testosterone secretion in normal adult men. J Clin Endocrinol Metab 42:112-116.

740 Ruder HJ, Loriaux DL, Lipsett MB (1972) Estrone sulphate production rate and metabolism in man. J Clin Invest 51:1020-1033.

741 Ruder HJ, Loriaux DL, Sherins RJ, Lipsett MB (1974) Leydig cell function in men with disorders of spermatogenesis. J Clin Endocrinol Metab 38:244-247.

742 Ruf KB, Younglai EV, Holmes MJ (1974) Induction of precocious sexual maturation in female rats by electrochemical stimulation of the brain. Brain Res 78:437-446.

743 Ruokonen A (1978) Steroid metabolism in steroid tissue: the metabolism of pregnenolone, pregnenolone sulfate, dehydroepiandrosterone and dehydroepiandrosterone sulfate in human and boar testes in vitro. J Steroid Biochem 9:939-946.

744 Ruokonen A, Laatikainen T, Laitinen EA, Vikko R (1972) Free and sulphate-conjugated neutral steroids in human testis tissue. Biochemistry 11:1411-1416.

745 Rush ME, Lipner H (1979) Blockade of gonadotropin-releasing hormone induced secretion of pituitary follicle-stimulating hormone by inhibin-containing preparations. Endocrinology 105:187-194.

746 Ruzicka L, Golderberg MW, Meyer J, Brüngger H, Eichenberger E (1934) Uber die Synthese des Testikelhormons (Androsteron) und stereosomerer desselben durch Abbau hydrierter sterine. Helv Chim Acta 17:1395-1406.

747 Ruzicka L, Wettstein A (1935) Uber die kunstliche Herstellung dis Testikelhormons Testosteron (Androsten-3-on-17-ol). Helv Chim Acta 18: 1264-1275.

748 Ryan KD, Foster DL (1980) Neuroendocrine mechanisms involved in onset of puberty in the female: concepts derived from the lamb. Fed Proc 39:2372-2377.

749 Ryan KJ, Naftolin R, Reddy V, Flores F, Petro Z (1972) Estrogen formation in the brain. Am J Obstet Gynecol 114:454-460.

750 Saez JM, Forest MG, Morera AM, Bertrand J (1972) Metabolic clearance rate and blood production rate of testosterone and dihydrotestosterone in normal subjects, during pregnancy and in hyperthyroidism. J Clin Invest 51:1226-1234.

751 Saez JM, Hauor F, Loras B, Sanchez P, Cathiard AM (1978) Oestrogen induced Leydig cell refractoriness to gonadotrophin stimulation. Acta Endocrinol 89: 379-392.

752 Sagel J, Distiller LA, Joffe A (1975) The role of luteinizing hormone-releasing hormone in the diagnosis of constitutional delayed puberty. Postgrad Med J 51:611-614.

753 Said SI, Porter JC, Vijayan E, Samson R, McCann SM (1978) VIP and hypothalamic-pituitary function. Scand J Gastroenterol 13:154.

754 Samson WK, Said SI, Graham JW, McCann SM (1978) Vasoactive interstitial polypeptide concentrations in median eminence of hypothalamus. Lancet 2: 901-902.

755 Samuels LT, Bussman L, Matsumoto K, Huseby RA (1975) Organization of androgen biosynthesis in the testis. J Steroid Biochem 6:291-296.

756 Samuels LT, Short GJ, Huseby RA (1964) The effect of diethylstilboestrol on testicular 17α-hydroxylase and 17-desmolase activities in BALB/c mice. Acta Endocrinol 45:487-497.

757 Samuels LT, Uchikawa T, Zain-ul-Abedin M, Huseby RA (1969) Effect of diethylstilboestrol on enzymes of cryptorchid mouse testis of BALB/c mice. Endocrinology 85:96-102.

758 Sanborn BM, Elkington SH, Chowdhury M, Tcholakian RK, Steinberger E (1975) Hormonal influences on the level of testicular androgen binding activity: effect of FSH following hypophysectomy. Endocrinology 96:304-312.

759 Sanborn BM, Steinberger A, Tcholakian RK, Steinberger E (1977) Direct measurement of androgen receptors in cultured Sertoli cells. Steroids 29:493-502.

760 Sandford LM, Winter JSD, Palmer WM, Howland BE (1974) The profile of LH and testosterone secretion in the ram. Endocrinology 95:627-631.

761 Sandler R, Hall PF (1966a) Stimulation in vitro by adenosine-3',5'-cyclic monophosphate of steroidogenesis in rat testes. Endocrinology 79:647-649.

762 Sandler R, Hall PF (1966b) The response of the rat testis to interstitial cell-stimulating hormone in vitro. Comp Biochem Physiol 19:833-843.

763 Santen RJ (1977) Independent effects of testosterone and oestradiol on the secretion of gonadotrophins in man. In: The testis in normal and infertile men. Eds: HR Nankin, P Troen. Raven Press, New York, pp197-211.

764 Santen RJ, Bardin CW (1973) Episodic luteinizing hormone secretion in man. J Clin Invest 52: 2617-2628.

765 Santen RJ, Ruby ER (1979) Enhanced frequency and magnitude of episodic luteinizing hormone-releasing hormone discharge as a hypothalamic mechanism for increased luteinizing hormone secretion. J Clin Endocrinol Metab 48:315-319.

766 Sarkar DK, Chiappa SA, Fink G (1976) Gonadotrophin-releasing hormone surge in pro-oestrous rats. Nature 264:461-463.

767 Sassin JF, Frantz AG, Kapen S, Weitzman ED (1973) The nocturnal rise of human prolactin is dependent on sleep. J Clin Endocrinol Metab 37:436-440.

768 Sassin JF, Parker DC, Mace JW, Gotlin RW, Johnson LC, Rossman LG (1969) Human growth hormone release: relation to slow wave sleep and sleep-waking cycles. Science 165:513-515.

769 Sauder SE, Corley KP, Hopwood NJ, Kelch RP (1981) Subnormal gonadotropin responses to gonadotropin-releasing hormone persist into puberty in children with isolated growth hormone deficiency. J Clin Endocrinol Metab 53:1186-1192.

770 Savard K, Goldzieher JW (1960) Biosynthesis of steroids in stallion testis tissue. Endocrinology 66:617-624.

771 Sawin CT, Ryan RJ, Longcope C, Fisher LE (1978) Effect of chronic administration of estrogen, androgen or both on serum levels of gonadotrophins in adult men. J Clin Endocrinol Metab 46:911-915.

772 Sawyer CH (1952) Stimulation of ovulation in the rabbit by the intraventricular injection of epinephrine or norepinephrine. Anat Rec 112:385-386.

773 Sawyer CH, Markee JE, Hollinshead WH (1947) Inhibition of ovulation in the rabbit by the adrenergic-blocking agent Dibenamine. Endocrinology 41:395-402.

774 Sawyer CH, Markee JE, Townsend BF (1949) Cholinergic and adrenergic components in the neurohumoral control of the release of LH in the rabbit. Endocrinology 44:18-37.

775 Saxena BB, Demura H, Gandy HM, Peterson RE (1968) Radioimmunoassay of human follicle stimulating and luteinizing hormones in plasma. J Clin Endocrinol Metab 28:519-534.

776 Saxena BB, Rathnam P (1971) Dissociation phenomenon and subunit nature of follicle stimulating hormone from human pituitary glands. J Biol Chem 246: 3549-3554.

777 Schalch DS, Parlow AF, Boon RC, Reichlin S (1968) Measurement of human luteinizing hormone in plasma by radioimmunoassay. J Clin Invest 47:665-678.

778 Schally AV, Arimura A, Redding T, Debeljuk L, Carter W, Dupont A, Vilchez-Martinez J (1976) Re-examination of plasma and bovine hypothalamic fractions for additional luteinizing hormone and follicle stimulating hormone-releasing activities. Endocrinology 98:380-391.

779 Schally AV, Redding TW, Arimura A, Dupont A, Linthicum GA (1977) Isolation of gamma-amino butyric acid from pig hypothalami and demonstration of its prolactin release-inhibiting (PIF) in vivo and in vitro. Endocrinology 100:681-691.

780 Schiff I, Wilson E, Newton R, Shane J, Kates R, Ryan KJ, Naftolin F (1976) Serum luteinizing hormone, follicle stimulating hormone and testosterone responses to gonadotrophin-releasing factor in males with varicocoeles. Fertil Steril 27:1059-1061.

781 Schöller R, Roger M, Leymarie P, Castanier M, Toublanc JE, Canlorbe P, Job JC (1975) Evaluation of Leydig cell function in normal prepubertal and pubertal boys. J Steroid Biochem 6:95-99.

782 Schonfeld WA (1943) Primary and secondary sexual characteristics. Study of their development in males from birth through maturity, with biometric study of penis and testes. Am J Dis Child 65: 535-549.

783 Schonfeld WA, Beebe GW (1942) Normal growth and variation in the male genitalia from birth to maturity. J Urol 48:759-777.

784 Scott RS, Burger HG (1980) Inhibin is absent from azoospermic semen of infertile men. Nature 285: 246-247.

785 Segal S, Polishuk WZ, Ben-David M (1976) Hyperprolactinaemic male infertility. Fertil Steril 27: 1425-1427.

786 Serio M, Calabresi E, Mannelli M, Pazzagli M (1980) Testicular secretion in mammals and in man. In: Animal Models in Human Reproduction. Eds: M Serio, L Martini. Raven Press, New York, pp121-133.

787 Setchell BP (1980a) The functional significance of the blood testis barrier. J Androl 1:3-10.

788 Setchell BP (1980b) Inhibin. In: Animal Models in Human Reproduction. Eds: M Serio, L Martini. Raven Press, New York, pp135-147.

789 Setchell BP, Davies RV, Main SJ (1977) Inhibin. In: The Testis Vol 4. Eds: AD Johnson, WR Gomes, NL Van Demark. Academic Press, New York, pp190-237.

790 Setchell BP, Sirinathsinghji DJ (1972) Antigonadotrophic activity in rete testis fluid, a possible "inhibin". J Endocrinol 53:LX-LXI.

791 Setchell BP, Waites GMH (1975) The blood-testis barrier. In: Male Reproductive System; Section 7, Endocrinology: Handbook of Physiology Vol V. Eds: DW Hamilton, RO Greep. American Physiological Society, Washington DC, pp143-172.

792 Shaar CJ, Clemens JA (1974) The role of catecholamines in the release of anterior pituitary prolactin in vitro. Endocrinology 95:1202-1212.

793 Sharma DC, Gabrilove JL (1971) Biosynthesis of testosterone and oestrogen in vitro by the testicular tissue from patients with Klinefelter's syndrome. Acta Endocrinol 66:737-744.

794 Sharpe RM (1976) hCG-induced decrease in availability of rat testis receptor. Nature 264:644-646.

795 Sharpe RM (1977) Gonadotropin-induced reduction in the steroidogenic responsiveness of the immature rat testis. Biochem Biophys Res Commun 76:957-962.

796 Sharpe RM (1982) The hormonal regulation of the Leydig cell. In: Oxford Rev Reprod Biol Vol 4, Ed: CA Finn. (In press).

797 Sharpe RM, Cooper I, Rommerts FFG (1981) Sertoli-Leydig cell communication via an LHRH-like factor. Nature 290:785-787.

798 Sharpe RM, Cooper I, Rommerts FFG (1982) The secretion, measurement and function of a testicular LHRH-like factor. Ann NY Acad Sci 383:272-292.

799 Sharpe RM, Fraser HM (1980a) Leydig cell receptors for luteinizing hormone releasing hormone and its agonists and their modulation by administration or deprivation of the releasing hormone. Biochem Biophys Res Commun 95:256-262.

800 Sharpe RM, Fraser HM (1980b) HCG stimulation of testicular LHRH-like activity. Nature 287:642-643.

801 Sherins RJ, Brightwell D, Sternthal PM (1977) Longitudinal analysis of semen of fertile and infertile men. In: The testis in normal and infertile men. Eds: P Troen, HR Nankin. Raven Press, New York, pp443-488.

802 Sherins RJ, Loriaux DL (1973) Studies on the role of sex steroids in the feedback control of FSH concentrations in men. J Clin Endocrinol Metab 36: 886-893.

803 Sheth AR, Mugatwala PP, Shah GV, Rao SS (1975) Occurrence of prolactin in human semen. Fertil Steril 26:905-907,

804 Shimazaki J, Oki Y, Kurihara H, Furuya N, Shida K (1973) Solubilization of testosterone 5α reductase from nuclear fraction of rat ventral prostate. Endocrinol Jpn 20:489-495.

805 Shome B, Parlow AF (1974) Human follicle stimulating hormone (hFSH): First proposal for the amino acid sequence of the α-subunit (hFSHα) and first demonstration of its identity with the α-subunit of human luteinizing hormone (hLHα). J Clin Endocrinol Metab 39:199-202.

806 Shome B, Parlow AF (1977) Human pituitary prolactin (hPRL): the entire linear amino acid sequence. J Clin Endocrinol Metab 45:1112-1115.

807 Short RV (1960) The secretion of sex hormones by the adrenal gland. Biochem Soc Symp 18:59-84.

808 Short RV (1974) Sexual differentiation of the brain of the sheep. In: Endocrinologie sexuelle de la periode périnale. Eds: MG Forest, J Bertrand. INSERM Vol 32, 121-142.

809 Short RV (1980) The hormonal control of growth at puberty. In: Growth in Animals. Ed: TLJ Lawrence. Butterworths, London, pp25-45.

810 Siiteri PK, MacDonald PC (1966) Placental estrogen biosynthesis during human pregnancy. J Clin Endocrinol Metab 26:751-761.

811 Siiteri PK, MacDonald PC (1973) Role of extra-glandular oestrogen in human endocrinology. In: Handbook of Physiology Section 7, Vol II. Eds: RO Greep, EB Astwood. American Physiological Society, Washington DC, pp615-630.

812 Sizonenko PC (1975) Endocrine laboratory findings in pubertal disturbance. Clin Endocrinol Metab 4: 173-206.

813 Sizonenko PC (1978) Endocrinology in preadolescents and adolescents I. Hormonal changes during normal puberty. Am J Dis Child 132:704-712.

814 Sizonenko PC, Paunier L (1975) Hormone changes in puberty III. Correlations of plasma dehydroepiandrosterone, testosterone, FSH, LH with stages of puberty and bone age in normal boys and girls and in patients with Addison's disease or hypogonadism or with premature or late adrenarche. J Clin Endocrinol Metab 41:894-904.

815 Sizonenko PC, Paunier L, Carmignac D (1976) Hormonal changes during puberty IV. Longitudinal study of adrenal androgen secretion. Horm Res 7:288-302.

816 Sizonenko PC, Rappaport R, Josso N, Dray F (1977) FSH II. Evidence for its mediating role on testosterone secretion in hypopituitarism. Acta Endocrinol 84:390-401.

817 Sjöberg B, de la Torre B, Hedman M, Falkay G, Diczfalusy E (1979) Circadian variation in systemic hormone levels in healthy men. J Endocrinol Metab 2:131-137.

818 Sklar CA, Kaplan SL, Grumbach MM (1980) Evidence for dissociation between adrenarche and gonadarche - studies in patients with idiopathic precocious puberty, gonadal dysgenesis, isolated gonadotrophin deficiency and constitutionally delayed growth and adolescence. J Clin Endocrinol Metab 57:548-556.

819 Slaunwhite Jr WR, Lockie GN, Bach N, Sandberg AA (1962) Inactivity in vivo of transcortin-bound cortisol. Science 135:1062-1063.

820 Slaunwhite Jr WR, Samuels LT (1956) Progesterone as a precursor of testicular androgens. J Biol Chem 220:341-352.

821 Smals AGH, Kloppenburg PWC, Benraad TJ (1976) Circannual cycle in plasma testosterone levels in man. J Clin Endocrinol Metab 42:979-982.

822 Smith ER, Damassa BA, Davidson JM (1977) Feedback regulation and male puberty: testosterone-luteinizing hormone relationships in the developing rat. Endocrinology 101:173-180.

823 Smith KD, Steinberger E (1977) What is oligospermia? In: The Testis in Normal and Infertile Men. Eds: P Troen, HR Nankin. Raven Press, New York, pp489-503.

824 Smith KD, Tcholakian RK, Chowdhury M, Steinberger E (1974) Rapid oscillations in plasma levels of testosterone luteinizing hormone and follicle stimulating hormone in men. Fertil Steril 25: 965-975.

825 Smith PE (1927) The disabilities caused by hypophysectomy and their repair. J A M A 88:158-161.

826 Smith PE (1930) Hypophysectomy and replacement therapy in the rat. Am J Anat 45:205-256.

827 Smith PE, Engle ET (1927) Experimental evidence regarding the role of the anterior pituitary in the development and regulation of the genital system. Am J Anat 40:159-217.

828 Sneddon A, Marrian GF (1963) Sulphation of oestrone in vitro by bovine adrenal tissue. Biochem J 86: 385-388.

829 Sniffen RC (1950) The testis I. The normal testis. Arch Pathol 50:259-284.

830 Southren AL, Gordon GG, Tochimoto S (1968) Further study of factors affecting the metabolic clearance rate of testosterone in man. J Clin Endocrinol Metab 28:1105-1112.

831 Southren AL, Gordon GG, Tochimoto S, Pinzon G, Lane DR, Stylpulkowski W (1967) Mean plasma concentration, metabolic clearance and basal plasma production rates of testosterone in normal young men and women using a constant infusion procedure: effect of time of day and plasma concentration on the metabolic clearance rate of testosterone. J Clin Endocrinol Metab 27:686-694.

832 Spencer RF (1946) The cultural aspects of eunuchism. Ciba Found Symp 8(7):406-420.

833 Spitz IM, Diamant Y, Rosen E, Bell J, David MB, Polishuk W, Rabinowitz D (1974) Isolated gonadotropin deficiency. N Engl J Med 290:10-15.

834 Spooner PM, Mainwaring WIP (1973) Possible relevance of the formation of 5β reduced steroids to haematopoesis in chick blastoderm. Acta Endocrinol (Suppl) 177:181.

835 Stearns EL, Winter JSD, Faiman C (1973) Positive feedback effect of progestin upon serum gonadotropins in estrogen-primed castrate men. J Clin Endocrinol Metab 37:635-638.

836 Steinach E (1940) Sex and life: forty years of biological and medical experiments. Viking Press, New York.

837 Steinberger A (1979) Inhibin production by Sertoli cells in culture. J Reprod Fertil (Suppl) 26:31-45.

838 Steinberger A, Ficher M, Steinberger E (1970) Studies of spermatogenesis and steroid metabolism in cultures of human testicular tissue. In: The Human Testis. Eds: E Rosemberg, CA Paulsen. Plenum Press, New York, pp333-352.

839 Steinberger A, Lindsey JN, Heindel JJ, Sanborn BM, Elkington JSH (1975) Isolation and culture of FSH-responsive Sertoli cells. Endocr Res Commun 2: 261-272.

840 Steinberger A, Steinberger E (1976) Secretion of an FSH-inhibiting factor by cultured Sertoli cells. Endocrinology 99:918-921.

841 Steinberger E (1971) Hormonal control of mammalian spermatogenesis. Physiol Rev 51:1-51.

842 Steinberger E, Duckett GE (1967) Hormonal control of spermatogenesis. J Reprod Fertil (Suppl) 2:75-87.

843 Steinberger E, Ficher M, Smith KD (1970) Relation of in vitro metabolism of steroids in human testicular tissue to histologic and clinical findings. In: The Human Testis. Eds: E Rosemberg, CA Paulsen. Plenum Press, New York, pp439-458.

844 Steinberger E, Root A, Ficher M, Smith KD (1973) The role of androgens in the initiation of spermatogenesis in man. J Clin Endocrinol Metab 37:746-751.

845 Steinberger E, Smith KD, Tcholakian RK, Chowdhury AK, Steinberger A, Ficher M, Paulsen CA (1974a) Steroidogenesis in human testis. In: Male Fertility and Sterility: Proceedings of the Serono Symposium Vol 5. Eds: RE Mancini, L Martini. Academic Press, New Yor, pp149-174.

846 Steinberger E, Steinberger A (1969) The spermatogenic function of the testis. In: The Gonads. Ed: KW McKerns. Appleton-Century-Crofts, New York, pp715-737.

847 Steinberger E, Steinberger A, Sanborn BM (1978) Molecular mechanisms concerned with hormonal control of the seminiferous epithelium. In: Recent Progress in Andrology. Eds: A Fabbrini, E Steinberger. Proceedings of Serono Symposium Vol 14. Academic Press, London, pp143-178.

848 Steiner RA, Bremner WJ (1981) Endocrine correlates of sexual development in the male monkey, Macaca fascicularis. Endocrinology 109:914-919.

849 Stephenson RP (1956) A modification of receptor theory. Br J Pharmacol 11:379-393.

850 Stewart-Bentley M, Odell W, Horton R (1974) The feedback control of luteinizing hormone in normal adult men. J Clin Endocrinol Metab 38:545-583.

851 Strickland AL, Nayfeh SN, French FS (1970) Conversion of cholesterol to testosterone and androstanediol in testicular homogenates of immature and mature rats. Steroids 15:373-387.

852 Strott CA, Yoshimi T, Lipsett MB (1969) Plasma progesterone and 17 hydroxyprogesterone in normal men and children with congenital adrenal hyperplasia. J Clin Invest 48:930-939.

853 Styne DM, Grumbach MM (1978) Puberty in the male and female: its physiology and disorders. In: Reproductive Endocrinology. Eds: SSC Yen, R Jaffe. WB Saunders, Philadelphia, pp189-240.

854 Suh HK, Frantz AG (1974) Size heterogeneity of human prolactin in plasma and pituitary extracts. J Clin Endocrinol Metab 39:928-935.

855 Swerdloff RS, Heber D (1981) Endocrine control of testicular function from birth to puberty. In: The Testis. Eds: H Burger, DM de Kretser. Raven Press, New York, pp107-126.

856 Swerdloff RS, Odell WD (1977) Modulating influences of FSH, GH and prolactin on LH-stimulated testosterone secretion. In: The testis in normal and

infertile men. Eds: P Troen, HR Nankin. Raven Press, New York, pp395-401.

857 Swerdloff RS, Walsh PC (1973) Testosterone and oestradiol suppression of LH and FSH in adult male rats: duration of castration, duration of treatment and combined treatment. Acta Endocrinol 73:11-21.

858 Swerdloff RS, Walsh PC (1975) Pituitary and gonadal hormones in patients with varicocoele. Fertil Steril 26:1006-1012.

859 Swerdloff RS, Walsh PC, Jacobs HS, Odell WD (1971) Serum LH and FSH during sexual maturation in male rat: effect of castration and cryptorchidism. Endocrinology 88:120-128.

860 Tait JF (1963) Review: the use of isotopic steroids for the measurement of production rates in vivo. J Clin Endocrinol Metab 23:1285-1297.

861 Tait JF, Burstein S (1964) In vivo studies of steroid dynamics in man. In: The Hormones, Physiology, Chemistry and Applications. Eds: G Pincus, KV Thimann, EB Astwood. Academic Press, New York, pp441-557.

862 Tait JF, Horton R (1964) Some theoretical considerations on the significance of the discrepancy in urinary and blood production rate estimates of steroid hormones particularly in those of testosterone in young women. Steroids 4:365-386.

863 Tait JF, Horton R (1966) The in vivo estimation of blood production and interconversion rates of androstenedione and testosterone and the calculation of their secretion rates. In: Steroid dynamics. Eds: G Pincus, T Nakao, JF Tait. Academic Press, New York, pp393-424.

864 Takahara J, Arimura A, Schally AV (1974) Suppression of prolactin release by a purified procine PIF preparation and catecholamines infused into a rat hypophyseal portal vessel. Endocrinology 95:462-465.

865 Takashashi Y, Kipnis DH, Daughaday WH (1968) Growth hormone secretion during sleep. J Clin Invest 47: 2079-2090.

866 Tamaoki BI (1973) General Review: Steroidogenesis and cell structure. Biochemical pursuit of sites of steroid biosynthesis. J Steroid Biochem 4:89-118.

867 Tanner JM (1962) Growth at adolescence. Blackwell Scientific Publications Oxford.

868 Tanner JM (1974) Sequence and tempo in the somatic changes in puberty. In: Control of the onset of puberty. Eds: MM Grumbach, GD Grave, FE Mayer. J Wiley & Sons, New York, pp448-470.

869 Tashjian Jr AH, Barowsky NV, Jansen DK (1971) Thyrotropin releasing hormone: direct evidence for stimulation of prolactin production by pituitary cells in culture. Biochem Biophys Res Commun 43: 516-523.

870 Taurog JD, Moore RJ, Wilson JD (1975) Partial characterization of the cytosol 3α-hydroxysteroid (NAD)P+ oxidoreductase of rat ventral prostate. Biochemistry 14:810-817.

871 Tea NT, Castanier M, Roger M, Schöller R (1975) Simultaneous radioimmunoassay of plasma progesterone and 17-hydroxyprogesterone. Normal values in children, men and women throughout the menstrual cycle and in early pregnancy. J Steroid Biochem 6:1509-1516.

872 Tetsuo M, Poth M, Markey SP (1982) Melatonin metabolite excretion during childhood and puberty. J Clin Endocrinol Metab 55:311-313.

873 Thamdrup E (1961) Precocious sexual development: a clinical study of 100 children. Thomas, Springfield, Illinois.

874 Thanki KH, Steinberger A (1976) ^{125}I-LH binding to rat testis at various ages and posthypophysectomy. Endocr Res Commun 3:49-62.

875 Thompson DW (trans) (1910) Historia Animalium. The works of Aristotle Vol IV. Eds: JA Smith, WD Ross. Clarendon Press, Oxford.

876 Thorner MO, Edwards CRW, Hanker JP, Abraham G, Besser GM (1977) Prolactin and gonadotropin interaction in the male. In: The testis in normal and infertile men. Eds: P Troen, HR Nankin. Raven Press, New York, pp351-366.

877 Thorner MO, Round J, Jones A, Fahmy D, Groom GV, Butcher S, Thompson K (1977) Serum prolactin and oestradiol levels at different stages of puberty. Clin Endocrinol 7:463-468.

878 Thorneycroft IH, Ribiero WO, Stone SC, Tillson SA (1973) A radioimmunoassay of androstenedione. Steroids 21:111-122.

879 Tiepolo L, Zuffardi O (1976) Localization of factors controlling spermatogenesis in the nonfluorescent portion of the human Y chromosome long arm. Human Genet 34:119-124.

880 de la Torre B, Norén S, Hedman M, Diczfalusy E (1978) Studies on the relationship between sperm count and steroid levels in spermatic and cubital veins of patients with varicocoele. Int J Androl 1:297-307.

881 Towobola DA, Crilly RC, Oakey RE (1980) Oestrone sulphate in plasma from postmenopausal women and the effects of oestrogen and androgen therapy. Clin Endocrinol 13:461-471.

882 Treloar OL, Wolf RC, Meyer RK (1972) Failure of a single neonatal dose of testosterone to alter ovarian finction in the Rhesus monkey. Endocrinology 90:281-284.

883 Tsai Y-H, Sanborn BM, Steinberger A, Steinberger E (1977) The interaction of testicular androgen-receptor complex with rat germ cell and Sertoli cell chromatin. Biochem Biophys Res Commun 75:366-372.

884 Tsou RC, Dailey RA, McLanahan CS, Parent AD, Tindall GT, Neill JD (1977) Luteinizing hormone releasing hormone (LHRH) levels in pituitary stalk plasma during the preovulatory gonadotrophin surge of rabbits. Endocrinology 101:534-539.

885 Tsuruhara T, Dufau ML, Cigorraga S, Catt KJ (1977) Hormonal regulation of testicular luteinizing hormone receptors. J Biol Chem 252:9002-9009.

886 Turek FW, Campbell CS (1979)Photoperiodic regulation of neuroendocrine-gonadal activity. Biol Reprod 20: 32-50.

887 Urban MD, Lee PA, Kowarski A, Plotnik LP, Migeon CJ (1979) Comparison of estimates of gonadotropin levels by isolated blood samples, integrated blood concentrations, and timed urinary fractions. J Clin Endocrinol Metab 48:732-735.

888 Vaitukaitis JL, Ross GT, Reichert Jr LE, Ward DN (1972) Immunologic basis for within and between species cross-reactivity of luteinizing hormone. Endocrinology 91:1337-1342.

889 Vale W, Burgus R, Guillemin R (1968) On the mechanism of action of TRF: effects of cycloheximide and actinomycin on the release of TSH stimulated in vitro by TRF and its inhibition by thyroxine. Neuroendocrinology 3:34-46.

890 Valk TW, Corley KP, Kelch RP, Marshall JC (1980) Hypogonadotrophic hypogonadism: hormonal responses to low dose pulsatile administration of gonadotropin-releasing hormone. J Clin Endocrinol Metab 51: 730-738.

891 Valladares LE, Payne AH (1979) Induction of testicular aromatization by luteinizing hormone in mature rats. Endocrinology 105:431-436.

892 van Beurden WMO, Roodnat B, de Jong FH, Mulder E, van der Molen HJ (1976) Hormonal regulation of LH stimulation of testosterone production in isolated Leydig cells of immature rats: the effect of hypophysectomy, FSH and oestradiol-17β. Steroids 28:847-866.

893 van Beurden WMO, Roodnat B, van der Molen HJ (1978) Effects of oestrogens and FSH on LH stimulation of steroid production by testis Leydig cells from immature rats. Int J Androl (Suppl) 2:374-383.

894 van der Molen HJ, Rommerts FFG (1981) Testicular steroidogenesis. In: The Testis. Eds: H Burger, DM de Kretser. Raven Press, New York, pp213-238.

895 van der Molen HJ, van der Vusse GJ, Cooke BA, de Jong FH (1975) Cellular and subcellular compartmentalization of steroid metabolism in the rat testis. J Reprod Fertil 44:351-362.

896 van der Vusse GJ, Kalkman ML, van der Molen HJ (1974) 3β hydroxysteroid dehydrogenase in rat testis tissue. Inter and subcellular localization and inhibition by cyanoke-tone and nagarse. Biochem Biophys Acta 348:404-414.

897 Vande Wiele RL, Ferin M (1974) The control of pulsatile gonadotrophin secretion. In: Chronobiological Aspects of Endocrinology. Eds: J Aschoff, F Ceresa, F Halberg. Symposia Medica Hoechst 9; Schattauer Verlag GmbH, Stuttgart, pp137-150.

898 Vande Wiele R, MacDonald P, Gurpide E, Lieberman S (1963) Studies on the secretion and interconversion of the androgens. Recent Prog Horm Res 19:275-310.

899 Vankirk K, Sassin JF (1969) Technique for serial blood sampling during sleep recording. Am J EEC Technol 9:143-146.

900 van Leeuwenhoeck A (1678) Observationes de natis e semine genitali animalculis. Philos Trans R Soc (Biol) 12:1040-1043.

901 Van Look PFA, Hunter WM, Corker CS, Bain DT (1977) Failure of positive feedback in normal men and subjects with testicular feminization. Clin Endocrinol 7:353-366.

902 Van Thiel DH, Sherins RJ, Myers Jr GH, Devita Jr VT (1972) Evidence for a particular seminiferous tubular factor affecting follicle stimulating hormone secretion in man. J Clin Invest 51:1009-19.

903 Van Zyl JA, Menkveld R, Kotze JJ van W, Retief AE, van Niekerk WA (1975) Oligozoospermia: a seven year survey of the incidence, chromosomal aberrations, treatment and pregnancy rate. Int J Fertil 20: 129-132.

904 Vaughan L, Carmel PW, Dyrenfurth I, Frantz AG, Antunes AL, Ferin M (1980) Section of the pituitary stalk in the Rhesus monkey. I. Endocrine studies. Neuroendocrinology 30:70-75.

905 Vaze AY, Thakur AN, Sheth AR (1980) Levels of inhibin in human semen and accessory reproductive organs. Andrologia 12:66-71.

906 Verhoeven G, Heyns W, De Moor P (1975) Testosterone receptors in the prostate and other tissues. Vitam Horm 33:265-281.

907 Vermeulen A (1977) Transport and distribution of androgens at different ages. In: Androgens and Antiandrogens. Eds: L Martini, M Motta. Raven Press, New York, pp53-65.

908 Vermeulen A, Rubens R, Verdonck L (1972)Testosterone secretion and metabolism in male senescence. J Clin Endocrinol Metab 34:730-735.

909 Vermeulen A, Stoica T, Verdonck L (1971) The apparent free testosterone concentration, an index of androgenicity. J Clin Endocrinol Metab 33: 759-767.

910 Vermeulen A, Verdonck L, Van der Straeten M, Orie N (1969) Capacity of the testosterone-binding globulin in human plasma and influence of specific binding of testosterone on its metabolic clearance rate. J Clin Endocrinol Metab 29:1470-1480.

911 Vigersky RA, Easley RB, Loriaux DL (1976) Effect of fluoxymesterone on the pituitary-gonadal axis. The role of testosterone-estradiol-binding globulin. J Clin Endocrinol Metab 43:1-9.

912 Vigersky RA, Kono S, Sauer M, Lipsett MB, Loriaux DL (1979) Relative binding of testosterone and oestradiol to testosterone-oestradiol binding globulin. J Clin Endocrinol Metab 49:899-904.

913 Vigersky RA, Loriaux DL, Howards SS, Hodgen GB, Lipsett MB, Chrambach A (1976) Androgen-binding proteins of testis epididymis and plasma in man and monkey. J Clin Invest 58:1061-1068.

914 Vijayan E, McCann SM (1978a) Reevaluation of the role of catecholamines in control of gonadotropin and prolactin release. Neuroendocrinology 25: 150-165.

915 Vijayan E, McCann SM (1978b) The effect of systemic administration of dopamine and apomorphin on plasma LH and prolactin concentrations in conscious rats. Neuroendocrinology 25:221-235.

916 Vilar O (1970) Histology of the testis from neonatal period to adolescence. In: The Human Testis. Eds: E Rosemberg, CA Paulsen. Plenum Press, New York, pp95-108.

917 von Baer KE (1827) De ovi mammalium et hominis genesi. Leopold Voss, Leipzig.

918 von Euler US, Hellner-Bjorkman S, Orwein I (1955) Diurnal variations in the excretion of free and conjugated noradrenaline and adrenaline in the urine from healthy subjects. Acta Physiol Scand 33, Suppl 118:10-16.

919 von Kölliker RA (1841) Beitrage zur Kenntnis der Geschlechtsverhaltnisse und der Samenflussigkeit wirbelloser Tiere. Berlin.

920 Waaler PE, Thorsen T, Stoa KF, Aarskog D (1974) Studies in normal male puberty. Acta Paediatr Scand (Suppl) 249:1-36.

921 Wachtel SS, Koo GC, Boyse EA (1975) Evolutionary conservation of H-Y ("male") antigen. Nature 254: 270-272.

922 Wakabayashi WM, Date Y, Tamaoki B (1973) On the mechanism of action of LHRF and PIF. Endocrinology 92:698-704.

923 Wallach EE, Decherney AH, Russ D, Duckett G, Garcia C-R, Root AW (1973) Episodic secretion of LH and FSH after ovariectomy. Obstet Gynecol 41:227-233.

924 Walsh EL, Cuyler WK, McCullagh DR (1934) Physiologic maintenance of male sex glands; effect of androtin on hypophysectomized rats. Am J Physiol 107:508-512.

925 Walsh PC, Madden JD, Harrod MJ, Godstein JL, MacDonald PC, Wilson JD (1974) Familial incomplete male pseudohermaphroditism type 2. Decreased dihydrotestosterone formation in pseudovaginal perineoscrotal hypospadius. N Engl J Med 291: 944-949.

926 Walsh PC, Swerdloff RS, Odell WD (1973) Feedback regulation of gonadotrophin secretion in men. J Urol 110:84-89.

927 Walton JS, Evins JD, Waites GMH (1978) Effect of chronic removal of testicular fluid on the release of follicle-stimulating hormone in hemi-castrated rams. J Endocrinol 77:421-422.

928 Wang C, Chan V, Yeung RTT (1978) Effect of surgical stress on pituitary-testicular function. Clin Endocrinol 9:255-266.

929 Wang CF, Lasley BL, Lein A, Yen SSC (1976) The functional changes of pituitary gonadotrophs during the menstrual cycle. J Clin Endocrinol Metab 42: 718-728.

930 Wang CF, Lasley BL, Yen SSC (1975) The role of oestrogen in the modulation of pituitary sensitivity to LRF (luteinizing hormone releasing factor) in men. J Clin Endocrinol Metab 41:41-43.

931 Wang C, Rebar RW, Hopper BR, Yen SSC (1980) Functional studies of the luteinizing hormone-Leydig cell-androgen axis: exaggerated response in C-18 and C-21 testicular steroids to various modes of luteinizing hormone stimulation. J Clin Endocrinol Metab 51:201-208.

932 Warne GL, Carter LN, Faiman C, Reyes FI, Winter JSD (1979) The relationship of adrenal androgens to the secretory patterns for cortisol, prolactin and growth hormone during early puberty. Pediatr Res 13:211-213.

933 Weddington SC, Hansson V, Ritzen EM, Hagenas L, French FS, Nayfeh SN (1975) Sertoli cell secretory function after hypophysectomy. Nature 254:148-146.

934 Weinberg U, Weitzman ED, Fukushima DK, Cancel GF, Rosenfeld RS (1980) Melatonin does not suppress the pituitary luteinizing hormone response to luteinizing hormone-releasing hormone in men. J Clin Endocrinol Metab 51:161-162.

935 Weinheimer B, Oertel GW, Leppla W, Blaise H, Bette L (1965) In: Androgens in normal and pathological conditions. Eds: A Vermeulen, D Exley. Excerpta Medica, Amsterdam, pp36-42.

936 Weinstein RL, Kelch RP, Jenner MR, Kaplan SL, Grumbach MM (1974) Secretion of unconjugated androgens and estrogens by the normal and abnormal human testis before and after human chorionic gonadotropin. J Clin Invest 53:1-6.

937 Weiss DB, Rodriquez-Rigau LJ, Smith KD, Steinberger E (1978) Leydig cell function in oligospermic men with varicocoele. J Urol 120:427-430.

938 Weitzman ED, Fukushima D, Nogeire C, Roffwarg H, Gallagher TF, Hellman L (1971) Twenty-four hour pattern of the episodic secretion of cortisol in normal subjects. J Clin Endocrinol Metab 33:14-22.

939 Welsh MJ, Wiebe JP (1975) Rat Sertoli cells: a rapid method for obtaining viable cells. Endocrinology 96:618-624.

940 Werder A, Illig R, Torresani T, Zachmann M, Baumann P, Ott F, Prader A (1976) Gonadal function in young adults after surgical treatment of cryptorchidism. Br Med J 2:1357-1359.

941 West CD, Damast BL, Sarro SD, Pearson OH (1956) Conversion of testosterone to estrogens in castrated, adrenalectomized human females. J Biol Chem 218: 409-418.

942 Wickings EJ, Usadel KH, Dathe G, Nieschlag E (1980) The role of follicle stimulating hormone in testicular function of the mature Rhesus monkey. Acta Endocrinol 95:117-128.

943 Wide L (1976) Human pituitary gonadotrophins. In: Hormone Assays and their Clinical Applications. Eds: JA Loraine, ET Bell. 4th edition; Churchill Livingstone, Edinburgh, pp87-140.

944 Wide L, Thoren L (1972) Increased urinary clearance of albumin, β2-microglobulin, insulin and luteinizing hormone following surgical or accidental trauma. Scand J Clin Lab Invest 30:275-281.

945 Wieland RG, De Covrey C, Levy RP, Zala AP, Hirschmann H (1965) $C_{19}O_2$ steroids and some of their precursors in blood from normal human adrenals. J Clin Invest 44:159-168.

946 Wieland RG, Zorn EM, Miles K, Hallberg MC (1974) Endocrinologic evaluation of oligospermic males: determination of dihydrotestosterone and oestradiol. Clin Res XXII:634A (Abstract).

947 Wildt L, Häusler A, Marshall G, Hutchison JS, Plant TM, Belchetz PE, Knobil E (1981) Frequency and amplitude of gonadotropin-releasing hormone stimulation and gonadotropin secretion in the Rhesus monkey. Endocrinology 109:376-385.

948 Wildt L, Marshall G, Knobil E (1980) Experimental induction of puberty in the infantile female Rhesus monkey. Science 267:1373-1375.

949 Wilson JD, Griffen JE, George FW (1980) Sexual differentiation: early hormone synthesis and action. Biol Reprod 22:9-17.

950 Winter JSD, Faiman C (1972) Pituitary-gonadal relations in male children and adolescence. Paediatr Res 6:126-135.

951 Winter JSD, Tararka S, Faiman C (1972) The hormonal response to HCG stimulation in male children and adolescents. J Clin Endocrinol Metab 34:348-353.

952 Winters SJ, Janick JJ, Loriaux DL, Sherins RJ (1979) Studies on the role of sex steroids in the feedback control of gonadotropin concentrations in men II. Use of the estrogen antagonist clomiphene citrate. J Clin Endocrinol Metab 48:222-227.

953 Wislocki GB, King LS (1936) The permeability of the hypophysis and the hypothalamus to vital dyes with a study of the hypophysial vascular supply. Am J Anat 58:421-472.

954 Wollensen F, Swerdloff RS, Odell WD (1976) LH and FSH responses to luteinizing-releasing hormone in normal, adult, human males. Metabolism 25:845-863.

955 Wong TW, Straus II FH, Warner NE (1973) Testicular biopsy in the study of male infertility I. Testicular causes of infertility. Arch Pathol 95: 151.

956 Woods MC, Simpson ME (1961) Pituitary control of the testis of the hypophysectomized rat. Endocrinology 69:91-125.

957 Wright K, Collins DC, Musey PI, Preedy JRK (1978) A specific radioimmunoassay for oestrone sulphate in plasma and urine without hydrolysis. J Clin Endocrinol Metab 47:1092-1098.

958 Yalow RS, Berson SA (1960) Immunoassay of endogenous plasma insulin in man. J Clin Invest 39:1157-1175.

959 Yamaji T, Ibayashi H (1969) Plasma dehydroepiandrosterone-sulphate in normal and pathological conditions. J Clin Endocrinol Metab 29:273-278.

960 Yanihara T, Troen P (1972a) Studies of the human testis I. Biosynthetic pathways for androgen formation in human testicular tissue in vitro. J Clin Endocrinol Metab 34:783-792.

961 Yanihara T, Troen P (1972b) Studies of the human testis II. A study of androstenediol and its monosulfate in human testes in vitro. J Clin Endocrinol Metab 34:793-800.

962 Yates FE (1981) Analysis of endocrine signals: the engineering and physics of biochemical communication systems. Biol Reprod 24:73-94.

963 Yen SSC (1977) Regulation of the hypothalamic-pituitary-ovarian axis in women. J Reprod Fertil 51:181-191.

964 Yen SSC (1980) The polycystic ovary syndrome. Clin Endocrinol 12:177-207.

965 Yen SSC, Lasley B, Wang C, Ehara Y (1975a) Steroid modulation of the hypothalamic-pituitary system in the secretion of reproductive hormones. J Steroid Biochem 6:1047-1053.

966 Yen SSC, Lasley BL, Wang CF, Leblanc H, Siler TM (1975b) The operating characteristics of the hypothalamic pituitary system during the menstrual cycle and observations of biological actions of somatostatin. Recent Prog Horm Res 31:321-364.

967 Yen SSC, Llerena O, Little B, Pearson OH (1968) Disappearance rates of endogenous luteinizing hormone and chorionic gonadotrophin in man. J Clin Endocrinol Metab 28:1763-1767.

968 Yen SSC, Llerena LA. Pearson OH, Little AS (1970) Disappearance rates of endogenous follicular stimulating hormone in serum following surgical hypophysectomy in men. J Clin Endocrinol Metab 30: 325-329.

967 Yen SSC, Tsai CC, Naftolin F, Vandenberg G, Ajabor L (1972a) Pulsatile patterns of gonadotropin release in subjects with and without ovarian function. J Clin Endocrinol Metab 34:671-675.

968 Yen SSC, Vandenberg G, Rebar R, Ehara Y (1972b) Gonadotropin dynamics in patients with gonadal dysgenesis: a model for the study of gonadotropin regulation. J Clin Endocrinol Metab 35:897-904.

969 Yen SSC, Vandenberg G, Tsai CC, Siler T (1974) Causal relationship between the hormonal variables in the menstrual cycle. In: Biorhythms and Human Reproduction. Eds: M Ferin, F Halberg, RM Richart, RL vande Wiele. Wiley, New York, pp203-218.

970 Yoshimoto Y, Moridera K, Imara H (1975) Restoration of normal pituitary gonadotropin reserve by administration of LHRH in patients with hypogonadotropic hypogonadism. N Engl J Med 292:242-245.

971 Zachmann M, Prader A, Kind HP, Haflinger H, Budliger H (1974) Testicular volume during adolescence. Cross-sectional and longitudinal studies. Helv Paediatr Acta 29:61-72.

972 Zanisi M, Motta M, Martini C (1973) Inhibitory effect of 5α-reduced metabolites of testosterone on gonadotrophin secretion. J Endocrinol 56:315-316.

973 Zarate A, Kastin AJ, Canales ES, Schally AV (1973) Effect of synthetic luteinizing hormone-releasing hormone (LH-RH) in two brothers with hypogonadotrophic hypogonadism and anosmia. J Clin Endocrinol Metab 36:612-614.

974 Zipf WR, Payne AH, Kelch RP (1978) Prolactin growth hormone and luteinizing hormone in the maintenance of testicular luteinizing hormone receptors. Endocrinology 103:595-600.

975 Zondek B, Aschheim S (1927) Hypophysenvorder lappen und ovarium. Beziehungen der endokrinen Drusen zur Ovarialfunktion. Arch f Gynak 130:1-45.

976 Longcope C, Tait JF (1971) Validity of metabolic clearance and interconversion rates of oestrone and 17β-oestradiol in normal male adults. J Clin Endocrinol Metab 32:481-490.

APPENDIX I

METHODOLOGY OF HORMONE ASSAYS

Gonadotrophins

Luteinizing hormone and follicle stimulating hormone were measured by double antibody radioimmunoassay (Hunter & Bennie, 1979). The standard for LH was MRC 68/40 (designated potency 77 u/ampoule) and FSH, MRC 69/104 (designated potency 10 u/ampoule) from the National Institute for Biological Standards and Control, London. The highly specific antisera to h-LH (F87) and to h-FSH (M93) raised against the human pituitary fraction CPDS/6 from Professor W.R. Butt were used at a dilution of 1 in 400,000. ^{125}I-LH was prepared from h-LH preparation IRC-2 from Dr A.S. Hartree; ^{125}I-FSH was prepared from highly purified h-FSH supplied by Professor W.R. Butt. Separation of bound from free hormone-tracer was achieved by filtration automatically in the Kemtech 3000 automated system (Bagshawe, 1975). The within-assay variation for LH was 11.3%, 6.6% and 8.0% for values under 2.0, between 2.0-6.0 and over 6.0 u/l respectively. The within-assay variation for FSH was 7.1%, 5.2% and 6.7% for values under 2.0, between 2.0-4.0 and over 4.0 u/l respectively. The mean between-assay variation for LH was 9.74% for quality control samples with values between 2-12 u/l. The mean between-assay variation for FSH was 8.30% for quality control samples with values between 1.8-10 u/l. The limit of detection was 0.65 u/l for LH and 0.74 u/l for FSH.

Testosterone

Testosterone was measured by radioimmunoassay in plasma extracts (hexane-ether 4:1) without chromatography (Corker & Davidson, 1978). The antiserum was raised in goat against testosterone-3-(O-carboxymethyl)-oxime bovine serum albumin and was kindly donated by Dr S. Tillson. The antiserum showed a significant cross-reaction with 5 -dihydrotestosterone (23.9%). [1,2,6,7-^{3}H] testosterone (Amersham International Ltd, Amersham) with spec. act. 94 Ci/mmol was the tracer and dextran-coated charcoal was used to separate the bound from free hormone. The within-assay variation was 7.45%, 4.13% and 6.36% for samples with mean values of 76.8, 414 and 818 ng/dl respectively. The mean between-assay variation based on the same three quality control pools was 6.55%. The limit of detection of the assay was 8 pg per tube or 16 ng/dl.

Oestradiol

Oestradiol was measured by radioimmunoassay (Van Look et al, 1977) in plasma extracts (diethylether) using a highly specific antiserum raised against 17β-oestradiol-6-(O-carboxymethyl)-oxime-bovine serum albumin kindly donated by Dr P. Dean (Dean et al, 1971; Exley et al, 1971). [2,4,6,7-^{3}H] oestradiol (Amersham International Ltd, Amersham) with spec. act.91 Ci/mmol was used as tracer and the separation of bound from free hormone was achieved by the addition of dextran-coated charcoal

suspension. The mean between-assay variation was 10% in six plasma pools with concentrations between 28-405 pg/ml. The within-assay variation was 9.3%. The limit of detection of the assay was 10 pg/ml.

Oestrone sulphate and oestrone

The simultaneous measurement for oestrone sulphate and oestrone in the same plasma samples were developed by the author specifically for the studies described in Section C. The methodology and validation are therefore described in full. Radioimmunoassay of oestrone sulphate is carried out with a specific antiserum directed against oestrone released after acid solvolysis of plasma samples. This approach was chosen because of the difficulty in raising a satisfactory antiserum against oestrone sulphate due to the instability of the immunogen.

Radioactive oestrogens Oestrone-3-sulphate [6,7-^{3}H] potassium salt (specific activity of 39 Ci/mmol or 101 mCi/mg) and oestrone [2,4,6,7-^{3}H] (specific activity of 110 Ci/mmol or 407 mCi/mg) from the Radiochemical Centre, Amersham, were used without further purification.

Non-radioactive oestrogens Oestrone-3-sulphate potassium salt containing 9.6% by weight of potassium acetate stabilizer was obtained from Sigma (London) Ltd, Poole. The purity was confirmed by thin-layer chromatography before use. Solutions were made up in phosphate-gelatin buffer and used within one week. Solutions of oestrone (Calbiochem C.P. Laboratories Ltd, Bishop

Stortford) were made up in absolute ethanol and stored at 4°C.

Solvents and reagents Organic solvents were AR grade (BDH Ltd, Poole). Diethyl ether was washed with acidified ferrous sulphate solution (50 G ferrous sulphate in 100 ml 5% w/v sulphuric acid) and redistilled before use. Sterile distilled water was purchased from Antigen Ltd, Rosecrea, Ireland. Steroid-free plasma was prepared by Charcoal Norit A (Sigma) adsorption of plasma obtained from female blood-donors.

Phosphate gelatin buffer (pH7) was prepared by dissolving NaN_3 (5 G), NaCl (45 G), Na_2HPO_4 (43 G), $NaH_2PO_4H_2O$ (27 G) and gelatin (5 g) in deionized distilled water (5 l). Dextran coated charcoal suspension contained 250 mg charcoal Norit A (Sigma) and 25 mg dextran T70 (Pharmacia G.B. Ltd, Hounslow) in 100 ml phosphate gelatin buffer.

Internal standards for estimation of procedural loss [^{3}H]oestrone sulphate (1000 CPM, 8 pg) was added to each plasma sample for assessment of procedural loss. For the oestrone estimations, three to four samples containing 1 ml of male plasma and [^{3}H] oestrone (5000 counts/min, 10 pg) were processed in each assay for estimation of group recovery. The mean recovery in oestrone sulphate samples (n = 173) was 63.4 ± 8.8% and oestrone (8 assays, n = 30) 84.3 ± 5.6%. After the addition of these internal standards, the samples were left at room temperature for 1 h to equilibrate.

Extraction of unconjugated oestrone prior to acid solvolysis One ml of plasma was shaken twice with 5 ml of freshly distilled peroxide-free diethyl ether. The organic phases were combined and dried under N_2 at 40°C. The residue was dissolved in 100 µl of phosphate gelatin buffer and kept at -20°C until assay.

Acid solvolysis This procedure was based on the method of Hawkins & Oak ey (1973) with minor modifications. After extraction of unconjugated oestrone, oestrone sulphate in each plasma sample was solvolysed by adding crystalline $(NH_4)_2$ SO_4 1 G, 66% aqueous (v/v) sulphuric acid 25 µl and ethyl acetate 5 ml. The reaction mixture was left overnight or for a minimum of 16 h in a water bath at 43°C. The unconjugated oestrone released by solvolysis was extracted into diethyl ether as above. The ether was washed with 1 ml of 8% (w/v) $NaHCO_3$, with 1 ml of sterile distilled water and evaporated to dryness. The residue was redissolved in 500 µl of phosphate gelatin buffer and kept at -20°C until assay. Two 100 µl aliquots from each sample were assayed and 200 µl counted for estimation of recovery.

Further purification of solvolysed extract by celite column chromatography Oestrone sulphate in plasma from post menopausal females and normal males were processed as described above and the oestrone obtained was assayed with and without purification with celite column chromatography (Thorneycroft, Ribiero, Stone & Tillson, 1973). Celite 545 (Johns-Manville Products Corporation, Richmond) was heated to 600°C for 18 hours to remove contaminants

and cooled in a dessicator. It was mixed thoroughly with ethylene glycol (2:1 W/V) and the columns packed dry in 5 ml graduated disposable pipettes to a height of 6 cm. After washing twice with 5 ml of iso-octane, the residues were applied to the columns in 0.5 ml iso-octane and then again washed with 2 ml and then 3.5 ml of iso-octane. The oestrone fraction was eluted with 4 ml of 15% ethyl-acetate in iso-octane which was evaporated under N_2. The residues were redissolved in 200 µl of buffer of which 100 µl was assayed and 50 µl counted for recovery.

Radioimmunoassay The antiserum was raised in ewes against oestone-6-(O-Carboxymethyl) oxime-bovine serum albumin (Martensz, Scaramuzzi & Van Look, 1979). Oestrone extracted before solvolysis and unconjugated oestrone liberated by solvolysis of the same samples were analysed together in one assay. To each sample antiserum (100 µl, 1:10,000 dilution in buffer) was added and the contents of the tubes mixed and equilibrated at room temperature for 1 h. [^{3}H]oestrone (5000 counts/min in 0.1 ml buffer) was added, mixed and the samples left overnight at 4°C. Unbound oestrogen was removed by adding 1 ml dextran coated charcoal suspension; the mixture was kept at 0°C for 15 min in an ice bath. The charcoal was separated by centrifugation at 1000 g for 10 min at 4°C and the supernatant fraction decanted into counting vials.

Standard curves were obtained from samples containing known quantities of oestrone (0-500 pg) processed by the same procedure as the unknown samples. The mass of oestrone in the latter was extrapolated

directly from the standard curves and corrected for procedural losses.

Liquid scintillation counting All samples were counted in 10 ml of scintillation fluid containing 2,5-Diphenyl-oxazole (10 G) and 1,4-di-2,5-phenylozazolyl-benzene (750 ng) in 2.5 litres sulphur-free toluene and 1.25 litres Triton X-100 (all from Koch-Light Laboratories Ltd, Colnbrook). Tritium was measured in a Packard Tri-Carb Liquid Scintillation spectrometer Model 3320 with a counting efficiency for tritium of 54%. Sufficient counts were recorded to give a counting error of less than 5%.

Specificity The specificity of the assay of oestrone sulphate was dependent on the solvolysis step and the specificity of the antiserum. Cross reactions with other steroids, expressed relative to oestrone (100%) at 50% tracer binding in the displacement of the dose-response curve (Abraham, 1969), was as follows: oestrone sulphate, 30.5% equilenin, 15.0% oestradiol-17β, oestradiol-17α, oestriol, dehydroepiandrosterone, testosterone, androsterone, 17α-hydroxyprogesterone, progesterone, cortisol and aldosterone, all less than 0.02%.

There was no significant difference in concentration of oestrone sulphate measured with and without celite column chromatography in replicate estimates of plasma from post-menopausal women (262 ± 43 vs 234 ± 33 pg/ml, $n = 8$, $p>0.1$) and normal men (722 ± 108 vs 674 ± 13 pg/ml, $n = 8$, $p>0.1$).

Accuracy Known amounts of oestrone sulphate (potassium

salt) and oestrone were added to water and samples of human plasma. The slopes of the regression equations were not significantly different from unity indicating that there were no systematic errors in the method. For oestrone sulphate added to water, amount found = 0.9946 (amount added) + 46.5 pg/ml; for oestrone sulphate added to plasma from post-menopausal women, amount found = 0.9631 (amount added) + 73.1 pg/ml. The γ intercept for oestrone sulphate added to water was not significantly higher than the average water blank in subsequent assays (20 pg/ml).

Precision The within-assay precision for the oestrone sulphate assay was estimated from assay of 25 duplicate samples (range of concentration 31-1588 pg/ml) in four assays was 6.7%. For the oestrone assay, the within-assay precision was determined in replicate samples at four different concentrations, 34 ± 2.4, 68 ± 4.7, 128 ± 11.9 and 264 ± 15.3 pg/ml, giving the coefficients of variations of 6.9, 6.9, 9.3 and 5.8% respectively with a mean of 7.2%.

Interassay precision was assessed by repeated analyses of three plasma samples with mean concentration (± SD) of 468 ± 44, 909 ± 105 and 2095 ± 502 pg/ml for oestrone sulphate and 33.3 ± 1.6, 43.3 ± 3.3 and 71.2 ± 17.9 pg/ml for oestrone in 3-5 consecutive assays. The average interassay coefficients of variation for the oestrone sulphate and the oestrone assays were 15% and 12.5% respectively.

Sensitivity The average binding in the zero standard tubes was 60 ± 3.9% of total oestrone tracer added (5000 CPM = 10.2 pg/tube) in eight consecutive assays. The sensitivity of the standard curve (3.4 pg/ml) defined as the smallest amount of steroid significantly different from zero was derived from the standard deviation of zero points of standard curves. Taking into account the procedural losses (mean recoveries for oestrone sulphate 63.4% and oestrone 84.3%) and the volume of plasma extract used for assay, the sensitivities of the assays were: oestrone sulphate 26.8 pg/ml; oestrone 4 pg/ml.

In the oestrone sulphate assay, the mean ± SD value for the water blank was 19.9 ± 4.16 pg/ml (n = 4) and average plasma blank, 39.8 ± 1.65 pg/ml (n = 3). The lower limit of detection of the method was arbitrarily set at 50 pg/ml, equivalent to 80% inhibition of the binding found at zero mass. The plasma and water blank values were consistently less than the detection limit and were not subtracted from the results.

In the oestrone assay, the mean (± SD) blank value for water was 8.3 ± 2.0 pg/ml (n = 5) and of oestrone free plasma was 30-206 pg/ml (n = 3). The lower limit of detection of the method was set at 15 pg/ml, equivalent to 70% inhibition of the binding at zero mass. Blank values were not subtracted from the results.

The specificity of the oestrone sulphate assay was achieved by the solvolysis procedure and the lack of cross reaction of the antiserum with possible interfering steroids. This was further substantiated by the

similarity of results obtained with or without celite column chromatography. The accuracy and precision were within the acceptable limits for steroid radioimmunoassays. The sensitivity was adequate for the range of values expected in the present studies and the values for water and steroid-free plasma were below the limits of detection of the method.

Androstenedione

Plasma androstenedione was measured by the radioimmunoassay method described by Baird et al (1974). The samples were purified by alumina column chromatography (McNatty et al, 1976). Intra- and inter-assay variations were 5.7% and 5.2% respectively and the limit of detection 145 pg/ml.

Prolactin

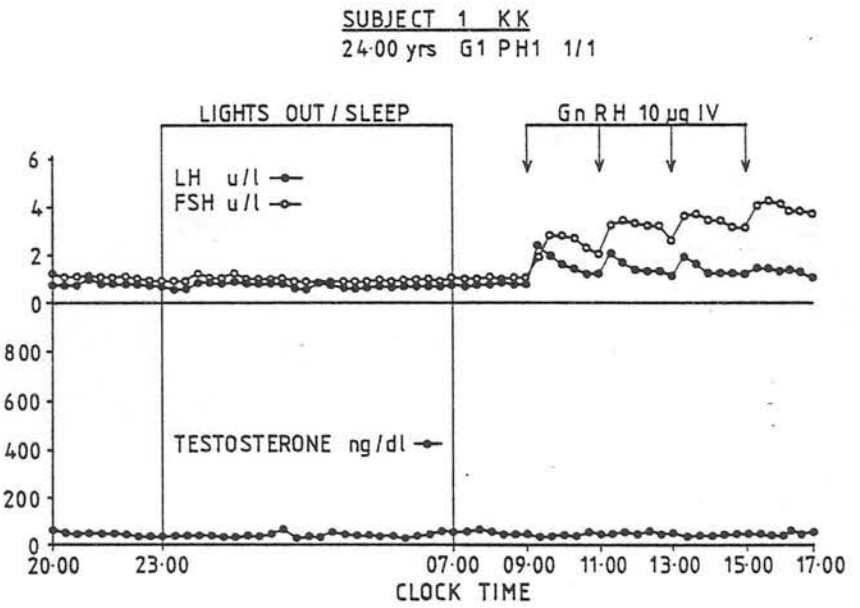
Prolactin was measured by double-antibody homologous radioimmunoassay (McNeilly & Hagen, 1974). Highly purified human prolactin (Dr H. Friesen) was used as standards and preparation of iodinated tracer. The antibody was raised in rabbits against an impure preparation of human prolactin. Due to the scarcity of pure preparations of human prolactin, standards for assay were prepared from doubling dilutions of pools of human serum containing high concentrations of prolactin standardized against Friesen's prolactin or MRC 71/222 (designated potency 10 mu/ampoule). Separation of bound from free hormone was accomplished by the addition of a

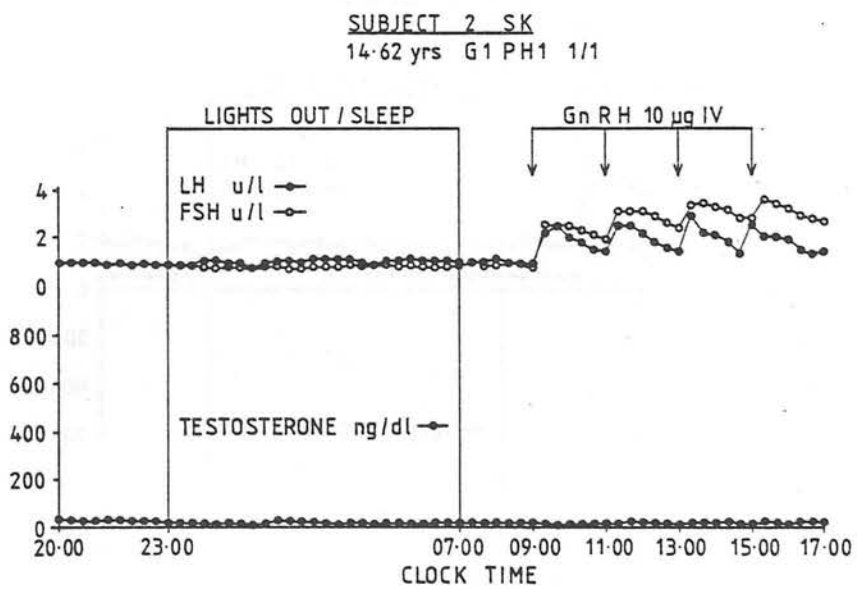
precipitating antirabbit gamma-globulin and the resulting precipitate separated by centrifugation. The within-assay variation based on ten replicates at three concentrations was 6.0%. The between-assay variation was 8.0%.

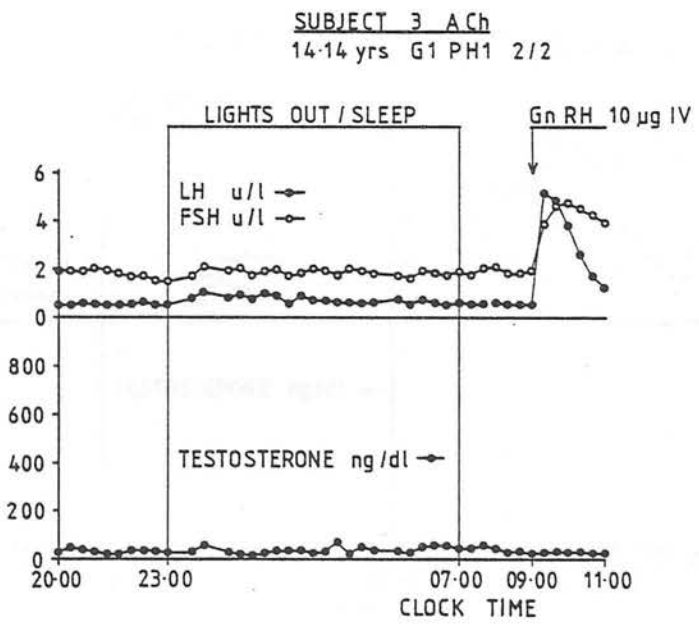
All hormone samples were assayed in duplicate and all samples from one study were measured in the same assay.

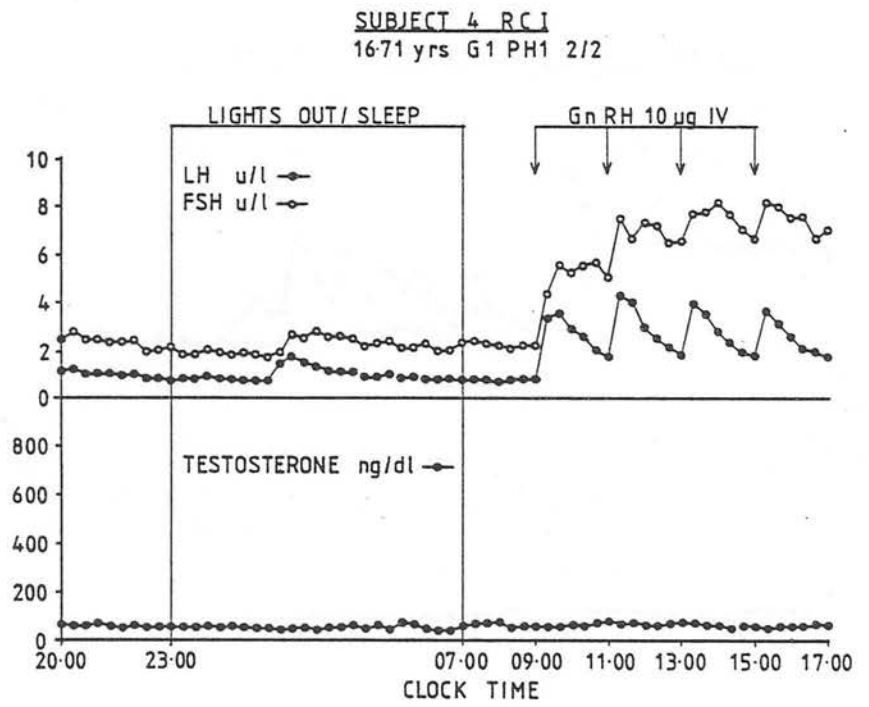
APPENDIX II

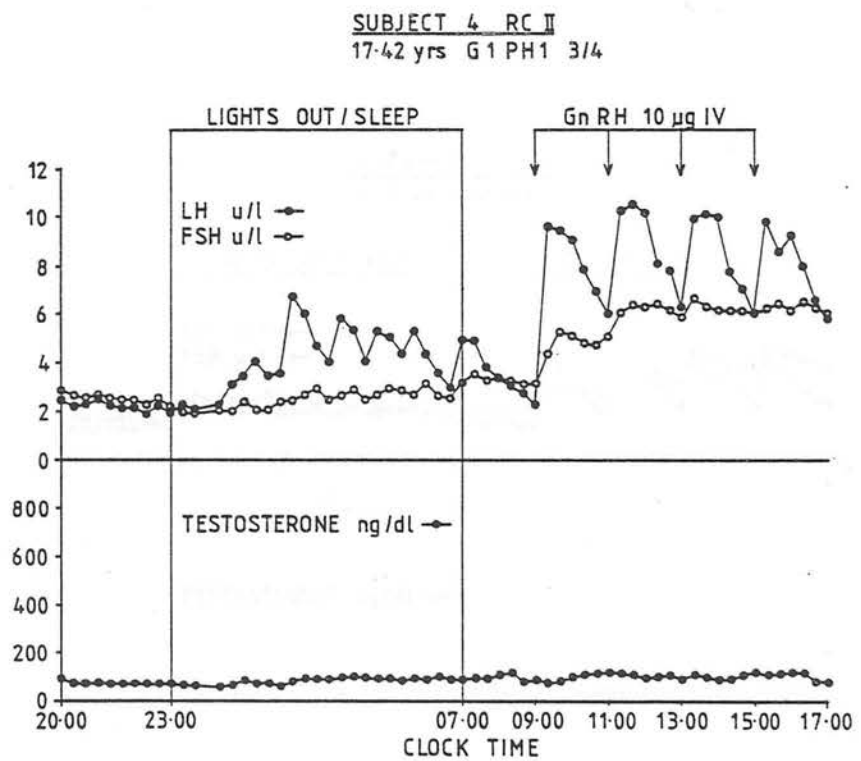
Plasma LH, FSH and testosterone concentrations at 20 minute intervals from 20.00-09.00 h and the response of these hormones to four 10 μg intravenous boluses of GnRH at two-hourly intervals in 33 studies from 16 adolescent subjects in order of increasing maturity. Two subjects (5 & 12) were studied on four occasions, three subjects (13, 14, 16) on three occasions and five subjects (4, 6, 8, 9, 11) on two occasions at approximately six-monthly intervals. All subjects slept during the dark period between 23.00-07.00 h undisturbed by blood sampling via an indwelling intravenous cannula with an extension into an adjacent room. Electroencephalographic monitoring of sleep onset and sleep stages was performed in 19 studies - see table 2.2. Puberty rating was determined according to Tanner (1962). Testicular volume in mls was measured by comparison with Prader's orchidometer. Subject 5 ST underwent left orchidopexy immediately prior to entering the study. He subsequently matured sexually and was studied on four occasions over a period of 26 months. However, since he received a twelve-week course of HCG (Pregnyl, Organon) after the first study, the hormonal data from the subsequent three studies had not been included in the group analyses. It is also noteworthy that the FSH concentrations in this patient were elevated only in late puberty in study IV. The left testis remained significantly smaller than the right.

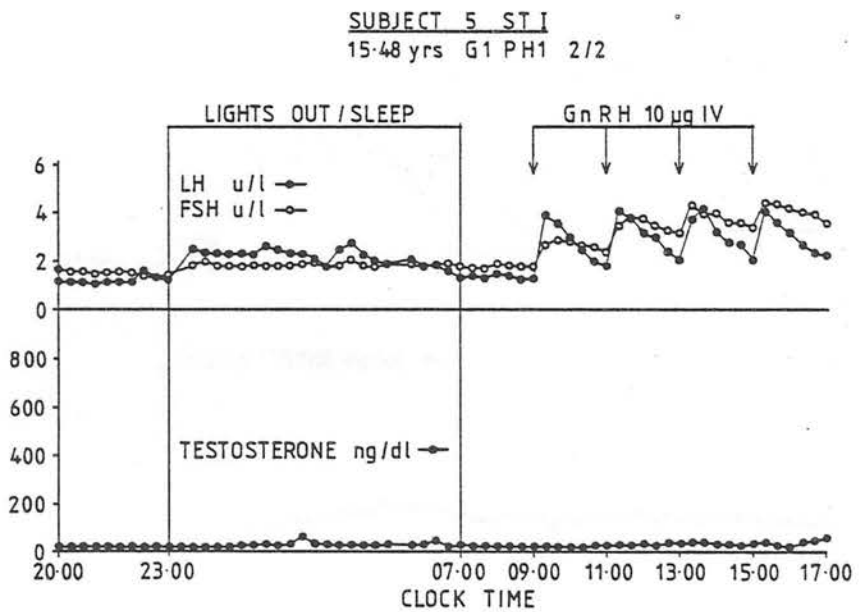

SUBJECT 1 KK
24·00 yrs G1 PH1 1/1
LIGHTS OUT / SLEEP
Gn R H 10 µg IV
LH u/l
FSH u/l
TESTOSTERONE ng/dl
6
4
2
0
800
600
400
200
0
20·00
23·00
07·00
09·00
11·00
13·00
15·00
17·00
CLOCK TIME

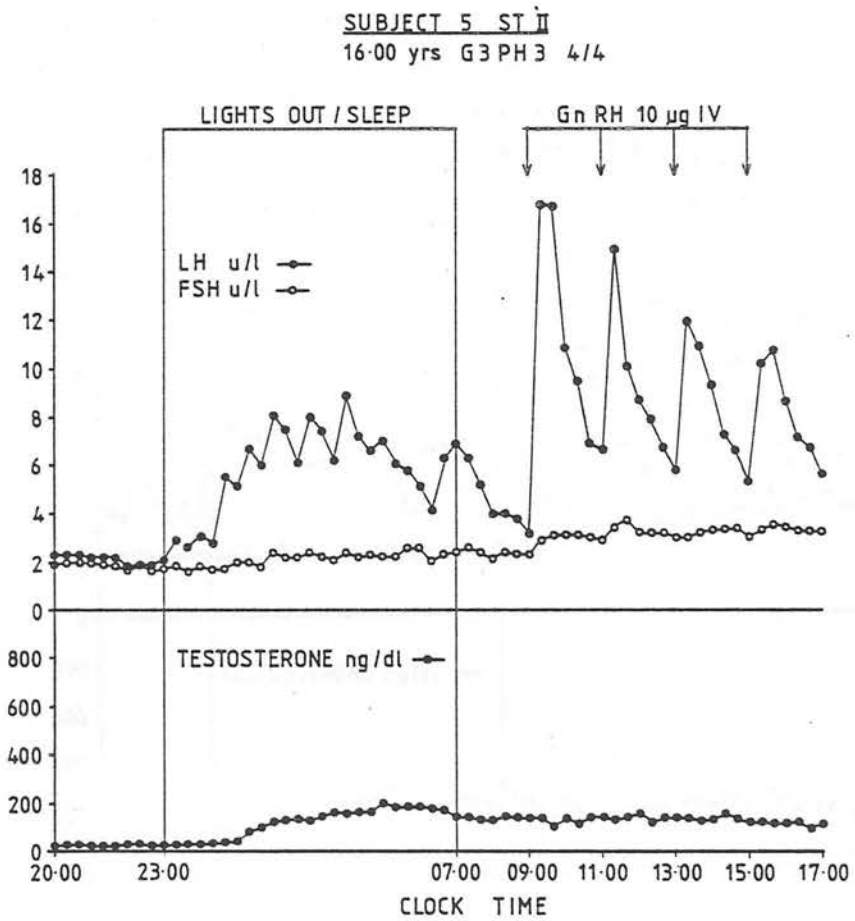

SUBJECT 2 SK
14·62 yrs G1 PH1 1/1
LIGHTS OUT / SLEEP
Gn R H 10 µg IV
LH u/l
FSH u/l
TESTOSTERONE ng/dl
4
2
0
800
600
400
200
0
20·00
23·00
07·00
09·00
11·00
13·00
15·00
17·00
CLOCK TIME

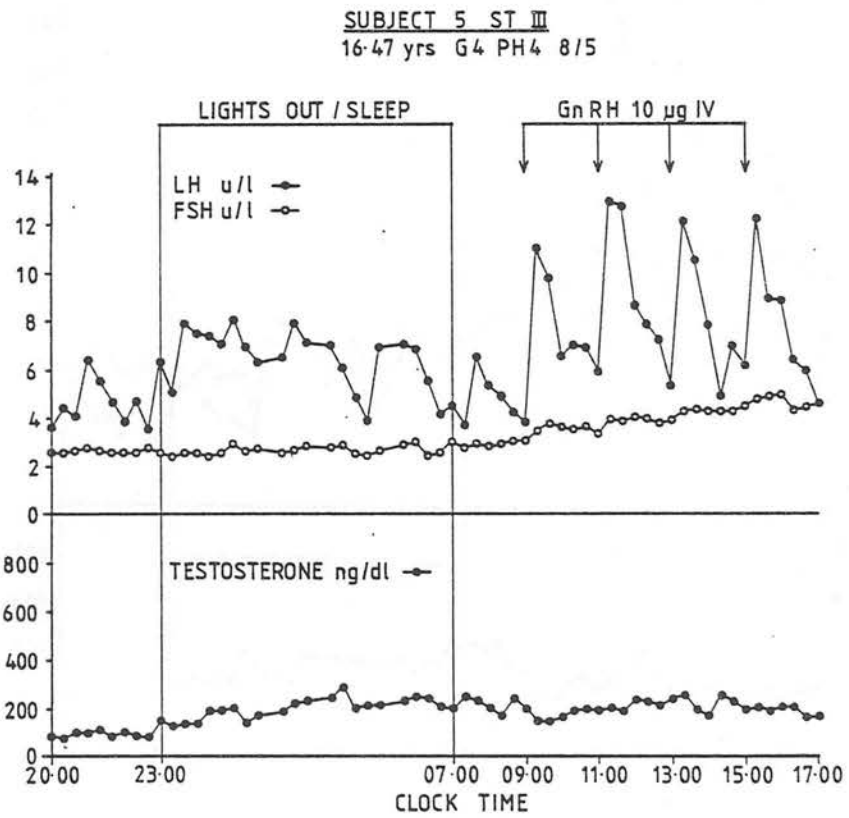

SUBJECT 3 A Ch
14·14 yrs G1 PH1 2/2
LIGHTS OUT / SLEEP
Gn RH 10 µg IV
LH u/l
FSH u/l
TESTOSTERONE ng/dl
6
4
2
0
800
600
400
200
0
20·00
23·00
07·00
09·00
11·00
CLOCK TIME

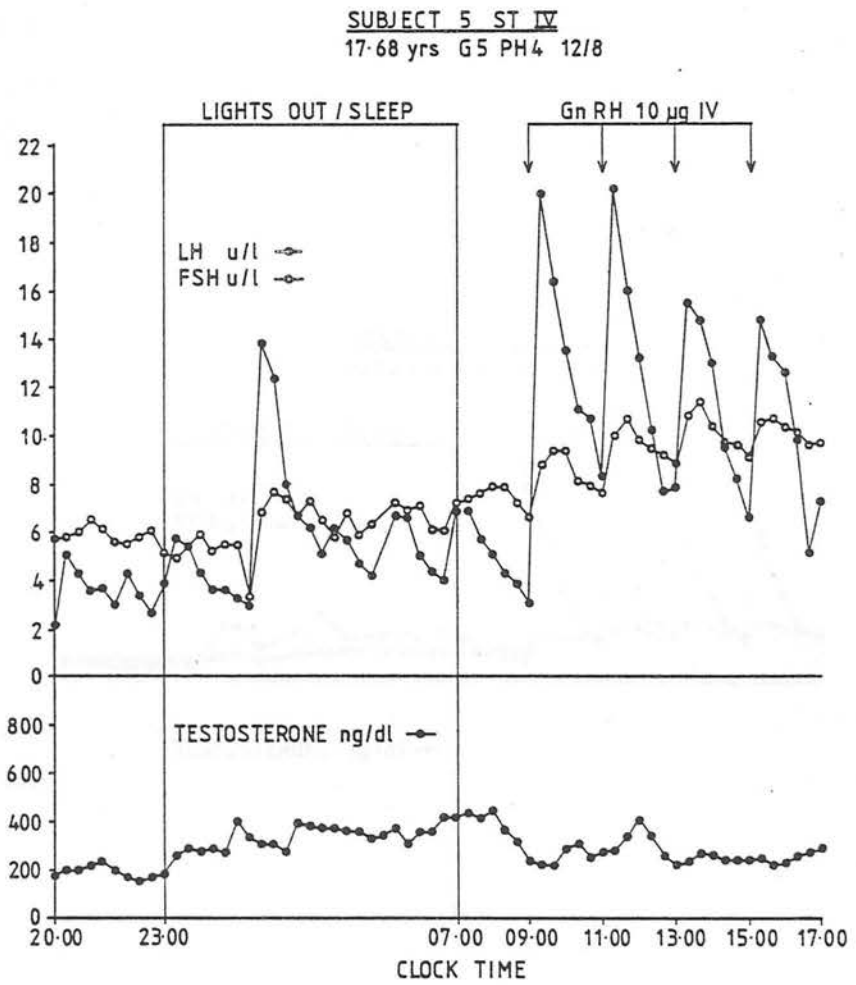

SUBJECT 4 RCI
16·71 yrs G1 PH1 2/2
LIGHTS OUT/ SLEEP
Gn RH 10 µg IV
LH u/l
FSH u/l
TESTOSTERONE ng/dl
10
8
6
4
2
0
800
600
400
200
0
20·00
23·00
07·00
09·00
11·00
13·00
15·00
17·00
CLOCK TIME

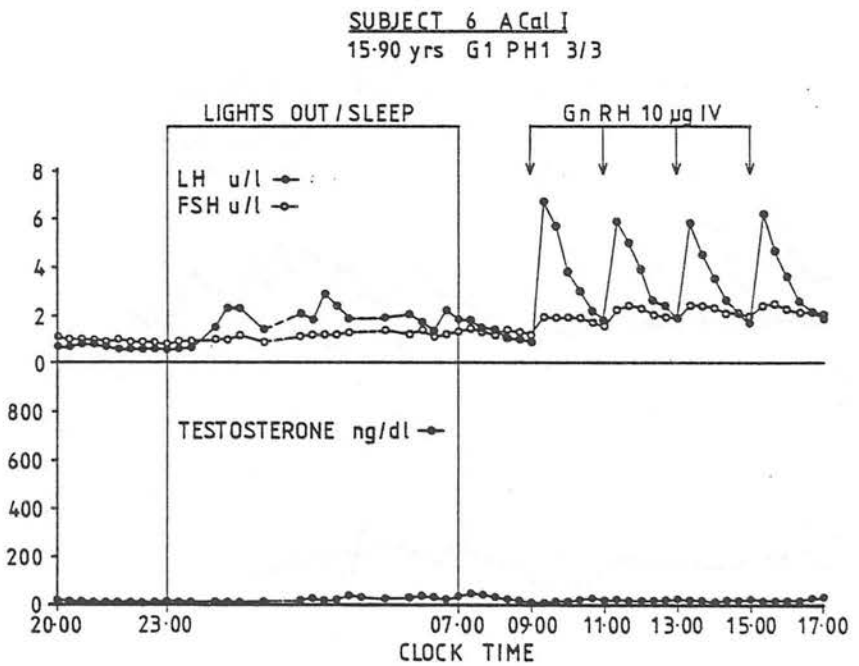

SUBJECT 4 RC II
17·42 yrs G 1 PH 1 3/4
LIGHTS OUT / SLEEP
Gn RH 10 µg IV
LH u/l
FSH u/l
TESTOSTERONE ng/dl
12
10
8
6
4
2
0
800
600
400
200
0
20·00
23·00
07·00
09·00
11·00
13·00
15·00
17·00
CLOCK TIME

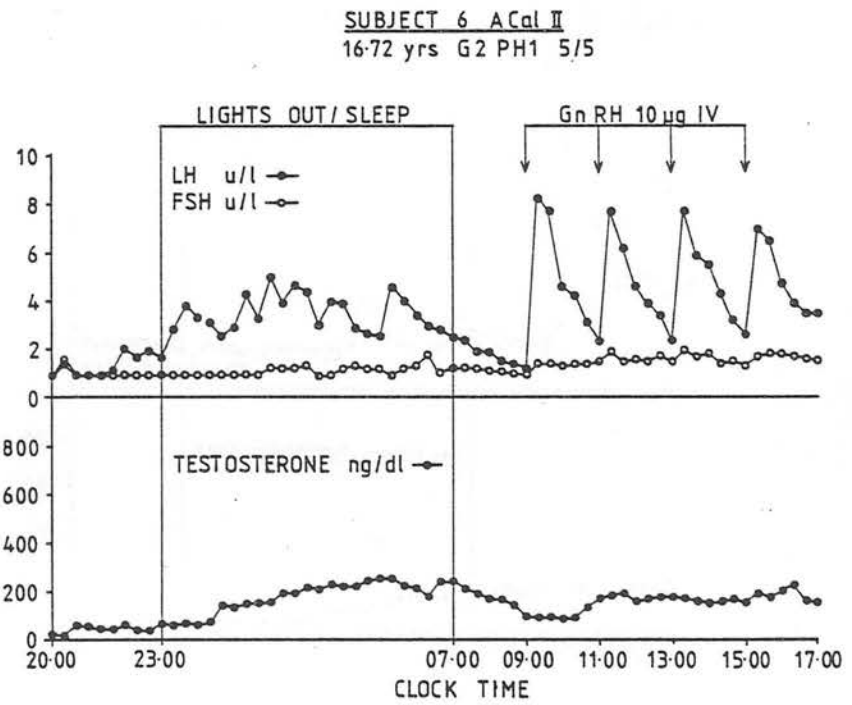

SUBJECT 5 ST I
15·48 yrs G1 PH1 2/2
LIGHTS OUT / SLEEP
Gn R H 10 µg IV
LH u/l
FSH u/l
6
4
2
0
800
600
400
200
0
TESTOSTERONE ng/dl
20·00
23·00
07·00
09·00
11·00
13·00
15·00
17·00
CLOCK TIME

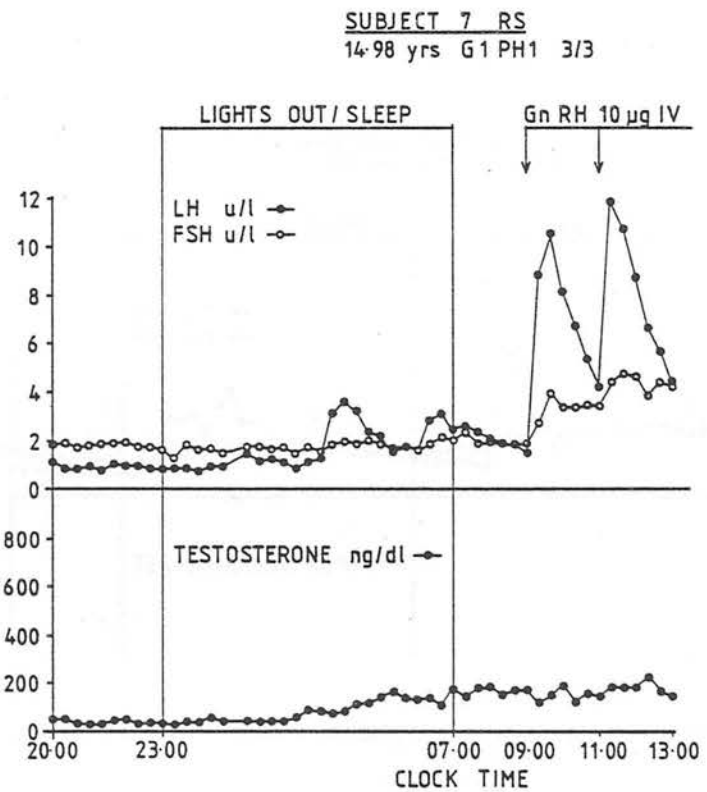

SUBJECT 5 ST II
16·00 yrs G3 PH3 4/4
LIGHTS OUT / SLEEP
Gn RH 10 µg IV
LH u/l
FSH u/l
TESTOSTERONE ng/dl
18
16
14
12
10
8
6
4
2
0
800
600
400
200
0
20·00
23·00
07·00
09·00
11·00
13·00
15·00
17·00
CLOCK TIME

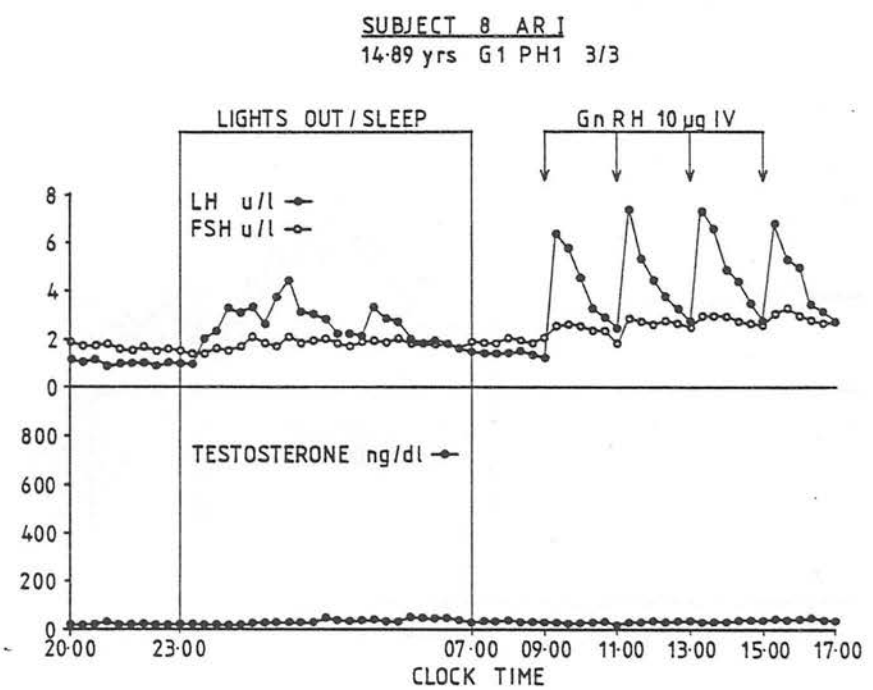

SUBJECT 5 ST III
16·47 yrs G4 PH4 8/5
LIGHTS OUT / SLEEP
GnRH 10 µg IV
LH u/l
FSH u/l
TESTOSTERONE ng/dl
14
12
10
8
6
4
2
0
800
600
400
200
0
20·00
23·00
07·00
09·00
11·00
13·00
15·00
17·00
CLOCK TIME

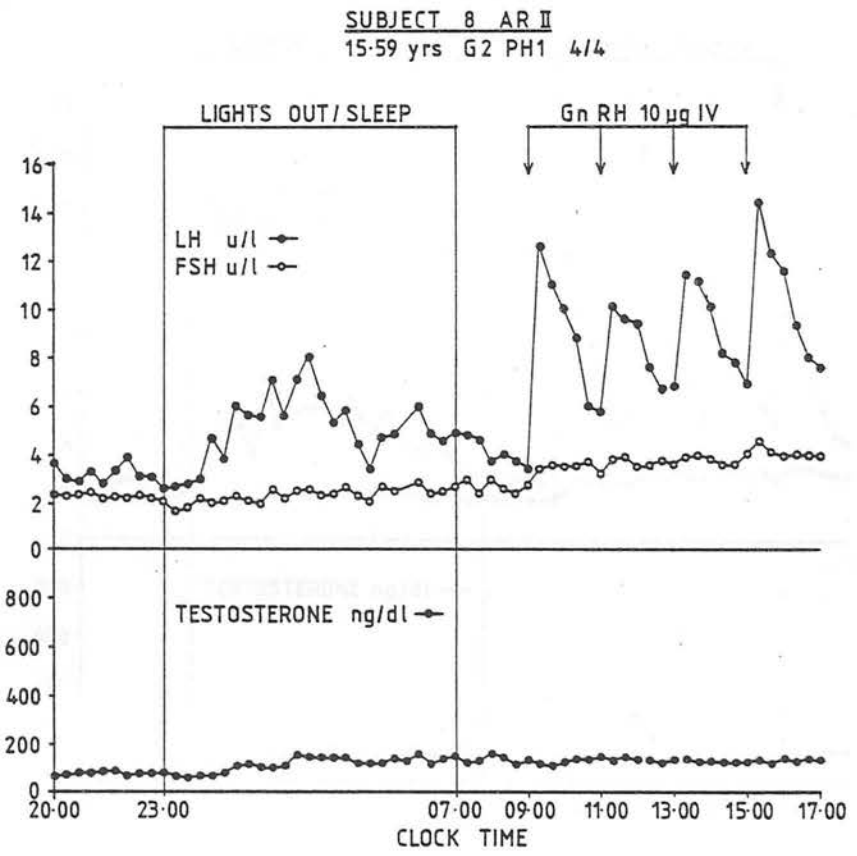

SUBJECT 5 ST IV
17·68 yrs G5 PH4 12/8
LIGHTS OUT / SLEEP
Gn RH 10 µg IV
LH u/l
FSH u/l
TESTOSTERONE ng/dl
22
20
18
16
14
12
10
8
6
4
2
0
800
600
400
200
0
20·00
23·00
07·00
09·00
11·00
13·00
15·00
17·00
CLOCK TIME

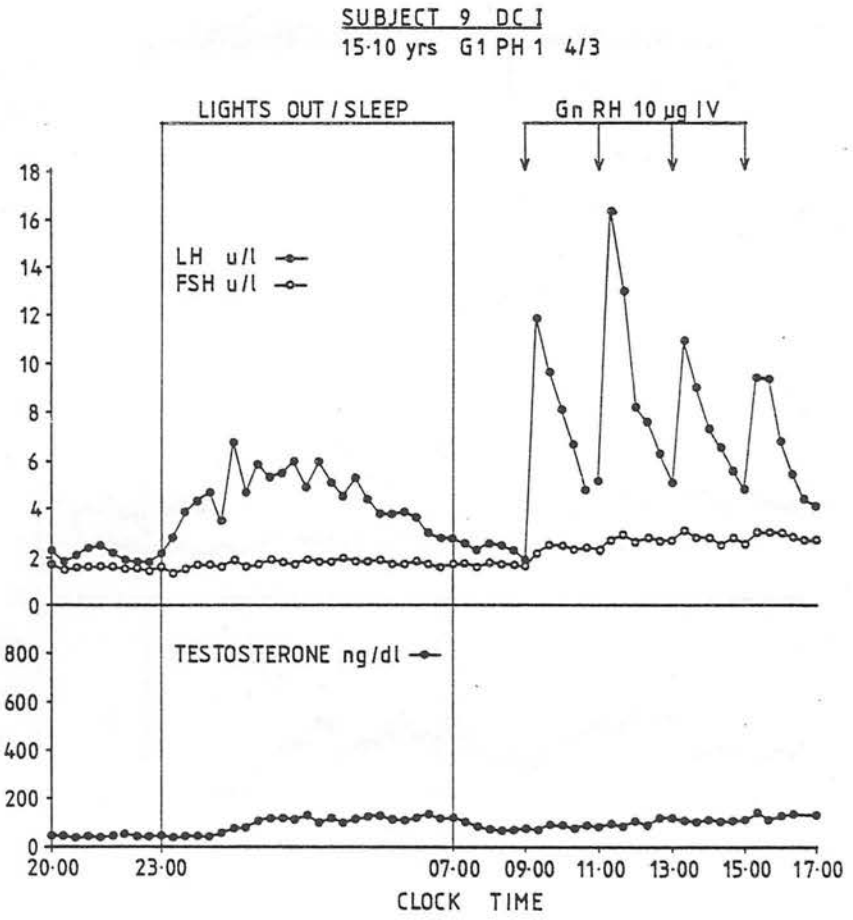

SUBJECT 6 A Cal I
15·90 yrs G1 PH1 3/3
LIGHTS OUT / SLEEP
Gn R H 10 µg IV
LH u/l
FSH u/l
TESTOSTERONE ng/dl
8
6
4
2
0
800
600
400
200
0
20·00
23·00
07·00
09·00
11·00
13·00
15·00
17·00
CLOCK TIME

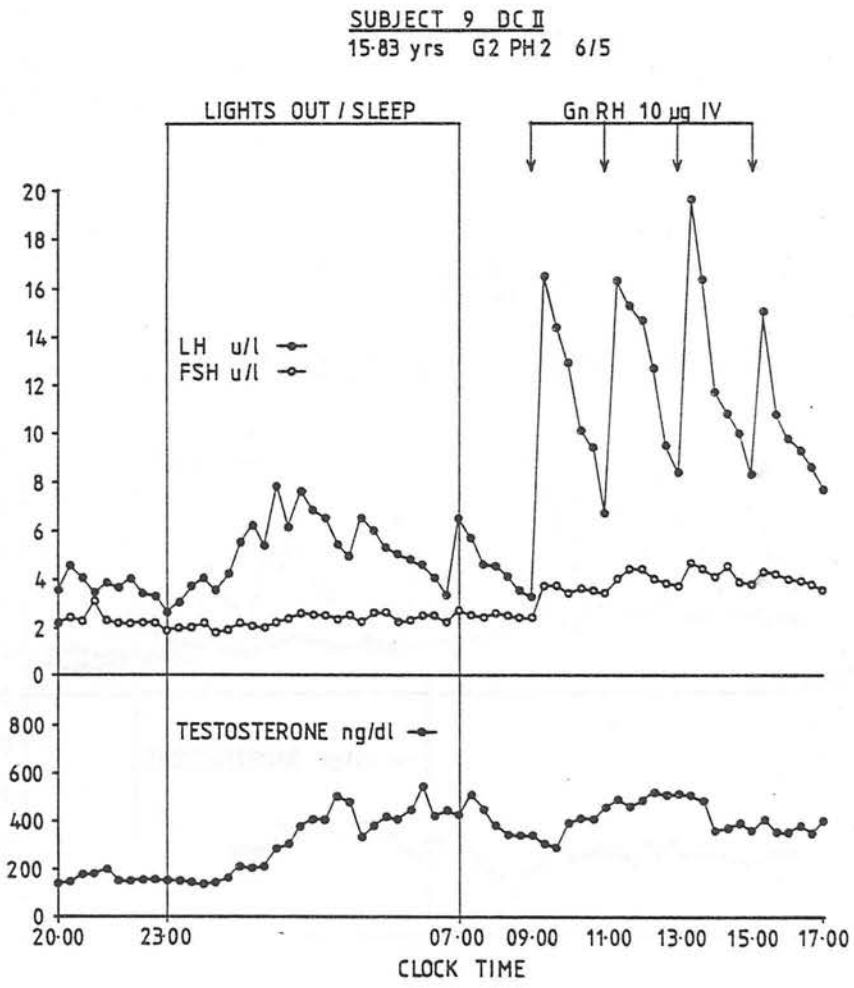

SUBJECT 6 ACal II
16·72 yrs G2 PH1 5/5
LIGHTS OUT / SLEEP
Gn RH 10 µg IV
LH u/l
FSH u/l
TESTOSTERONE ng/dl
10
8
6
4
2
0
800
600
400
200
0
20·00
23·00
07·00
09·00
11·00
13·00
15·00
17·00
CLOCK TIME

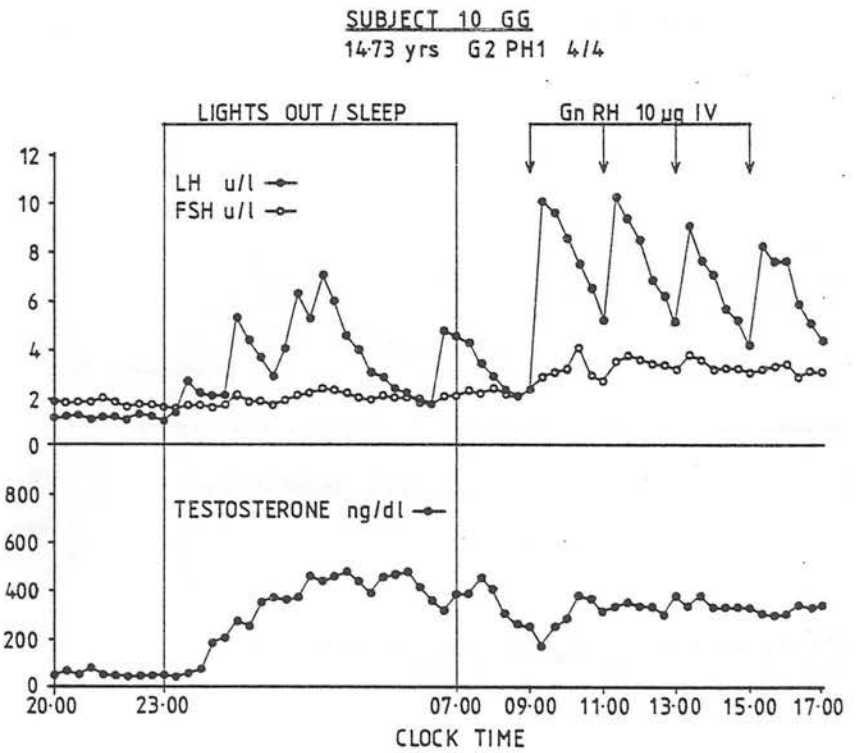

SUBJECT 7 RS
14·98 yrs G1 PH1 3/3
LIGHTS OUT / SLEEP
Gn RH 10 µg IV
LH u/l
FSH u/l
TESTOSTERONE ng/dl
12
10
8
6
4
2
0
800
600
400
200
0
20·00
23·00
07·00
09·00
11·00
13·00
CLOCK TIME

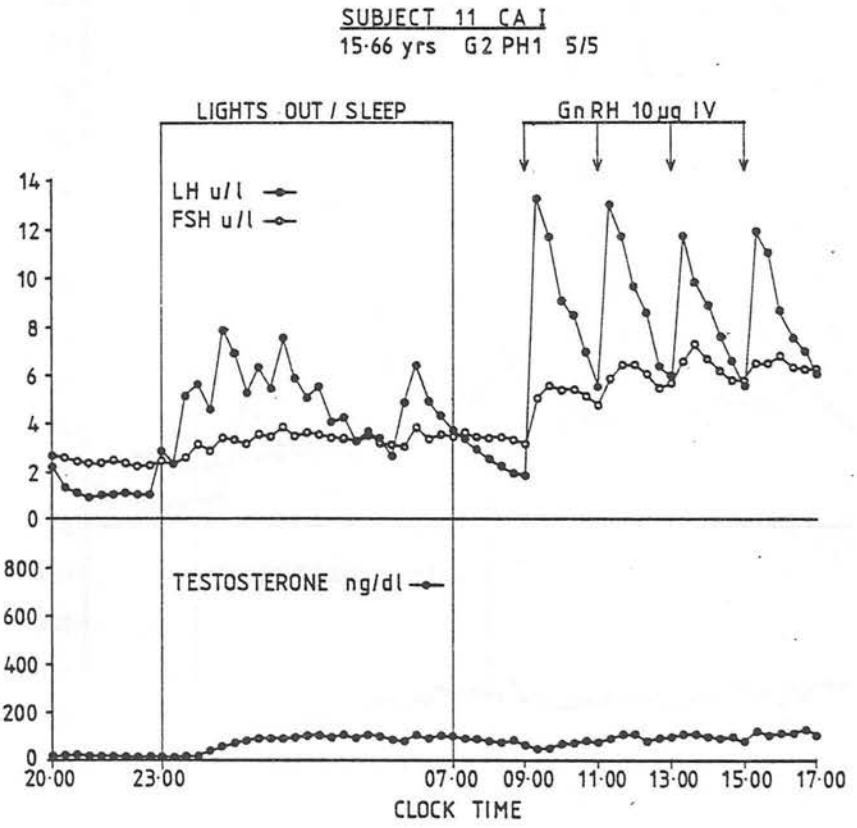

SUBJECT 8 AR I
14·89 yrs G1 PH1 3/3
LIGHTS OUT / SLEEP
Gn R H 10 µg IV
LH u/l
FSH u/l
TESTOSTERONE ng/dl
8
6
4
2
0
800
600
400
200
0
20·00
23·00
07·00
09·00
11·00
13·00
15·00
17·00
CLOCK TIME

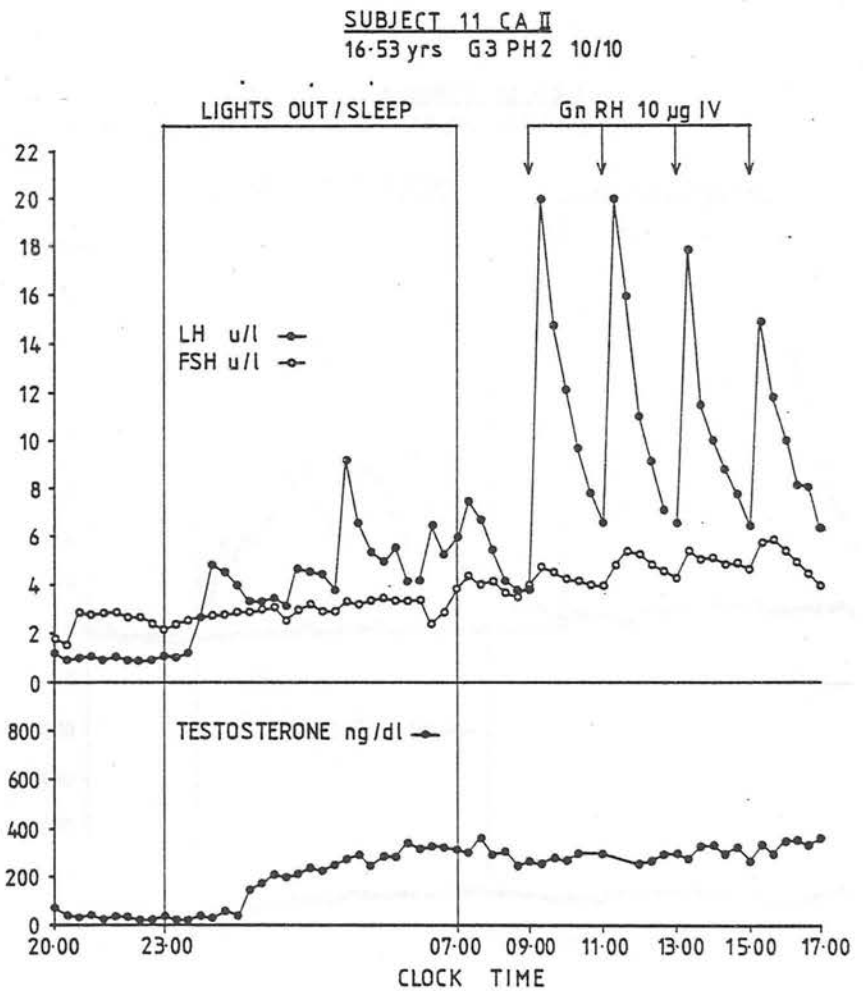

SUBJECT 8 AR II
15·59 yrs G2 PH1 4/4
LIGHTS OUT / SLEEP
Gn RH 10 µg IV
LH u/l
FSH u/l
TESTOSTERONE ng/dl
16
14
12
10
8
6
4
2
0
800
600
400
200
0
20·00
23·00
07·00
09·00
11·00
13·00
15·00
17·00
CLOCK TIME

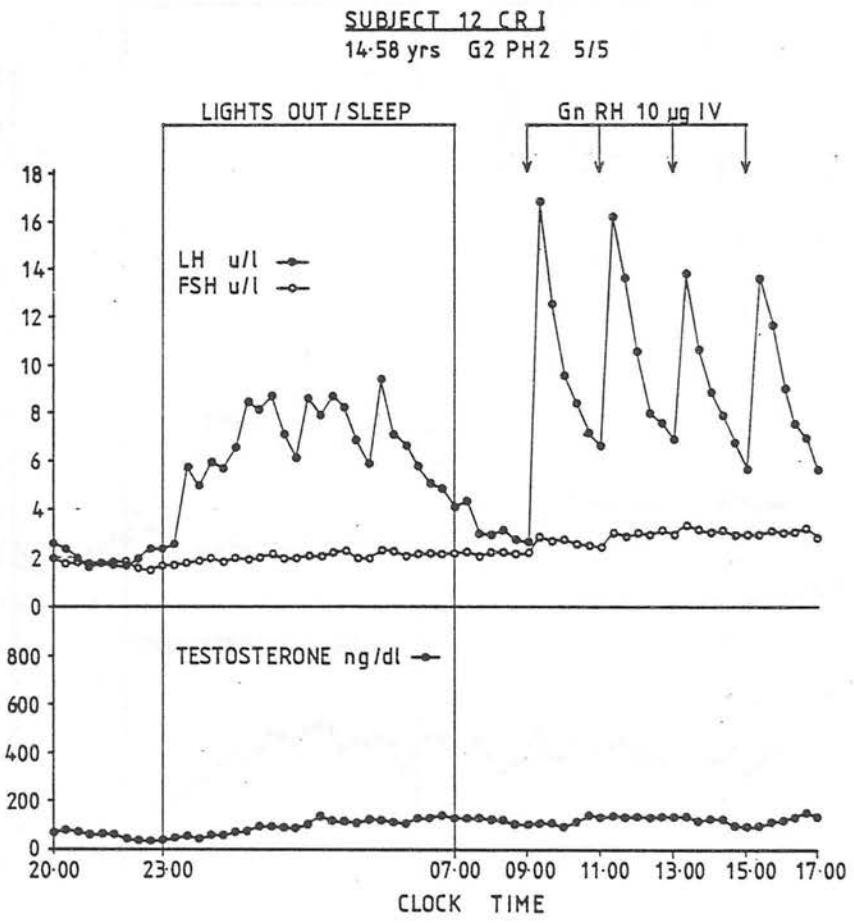

SUBJECT 9 DC I
15·10 yrs G1 PH 1 4/3
LIGHTS OUT / SLEEP
Gn RH 10 µg IV
LH u/l
FSH u/l
TESTOSTERONE ng/dl
18
16
14
12
10
8
6
4
2
0
800
600
400
200
0
20·00
23·00
07·00
09·00
11·00
13·00
15·00
17·00
CLOCK TIME

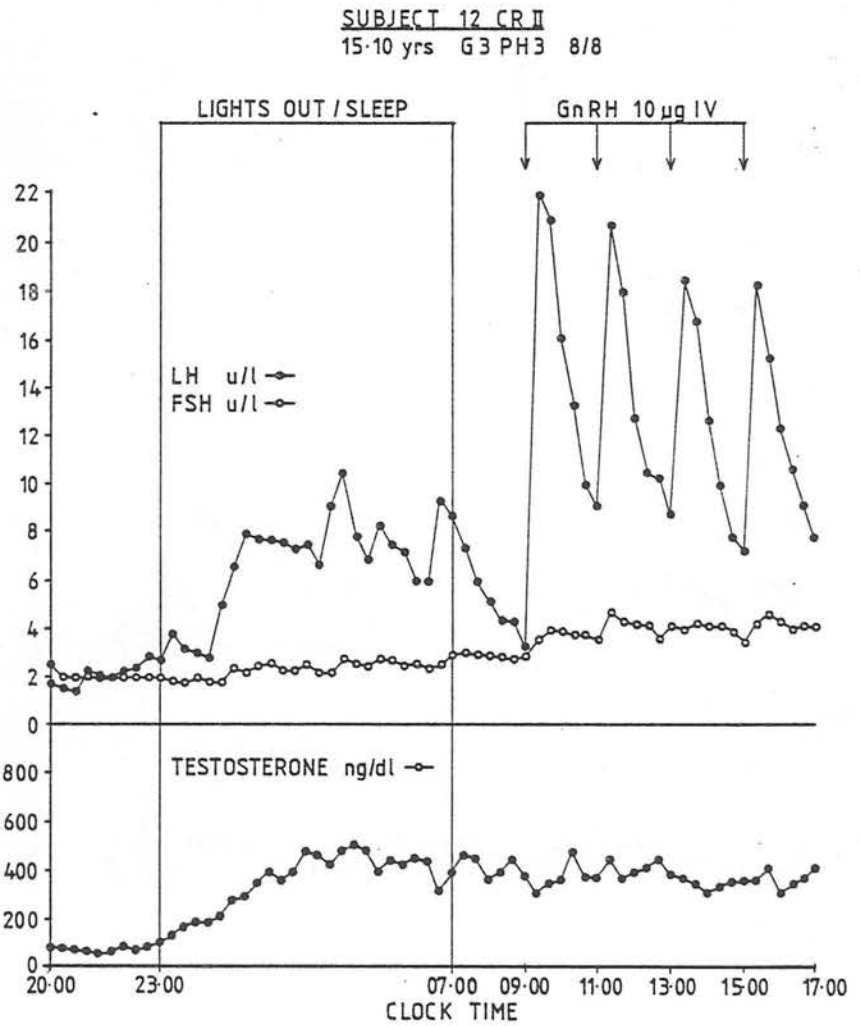

SUBJECT 9 DC II
15·83 yrs G2 PH2 6/5
LIGHTS OUT / SLEEP
Gn RH 10 µg IV
LH u/l
FSH u/l
TESTOSTERONE ng/dl
20·00
23·00
07·00
09·00
11·00
13·00
15·00
17·00
CLOCK TIME

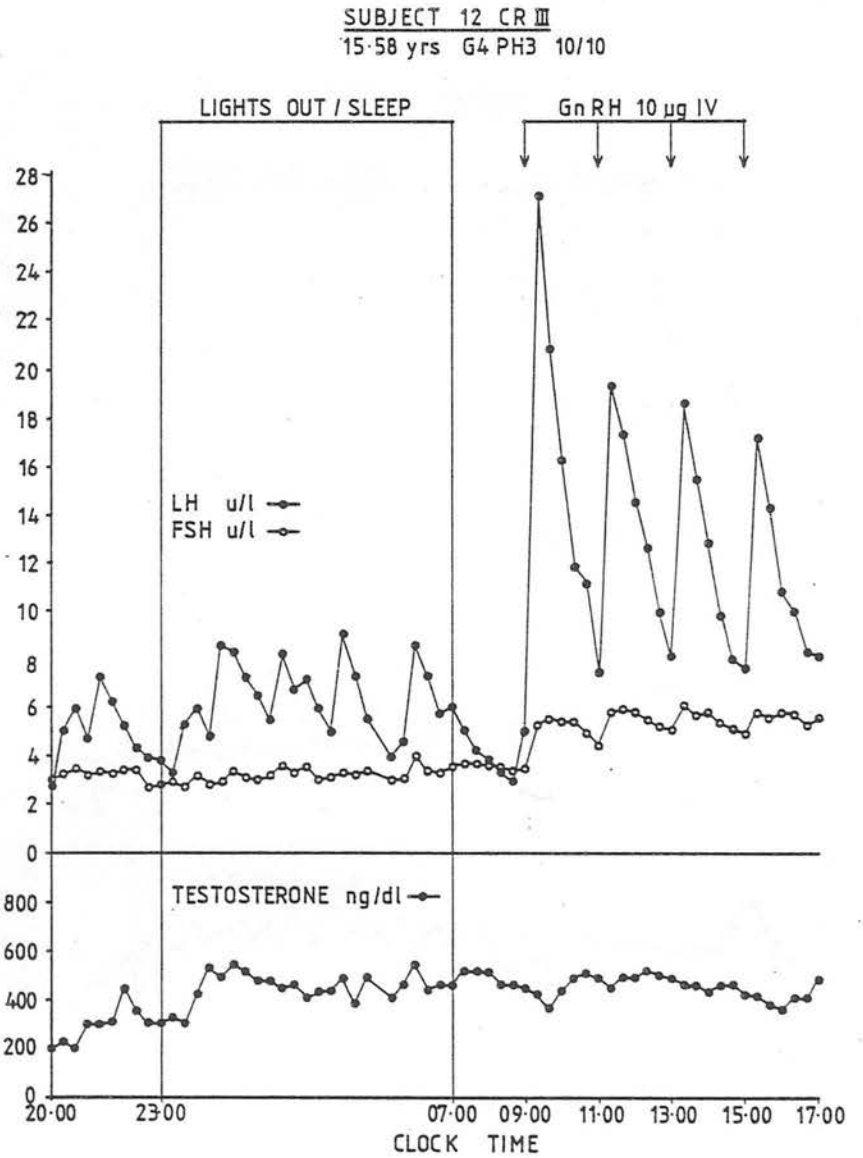

SUBJECT 10 GG
14·73 yrs G2 PH1 4/4
LIGHTS OUT / SLEEP
Gn RH 10 µg IV
LH u/l
FSH u/l
TESTOSTERONE ng/dl
12
10
8
6
4
2
0
800
600
400
200
0
20·00
23·00
07·00
09·00
11·00
13·00
15·00
17·00
CLOCK TIME

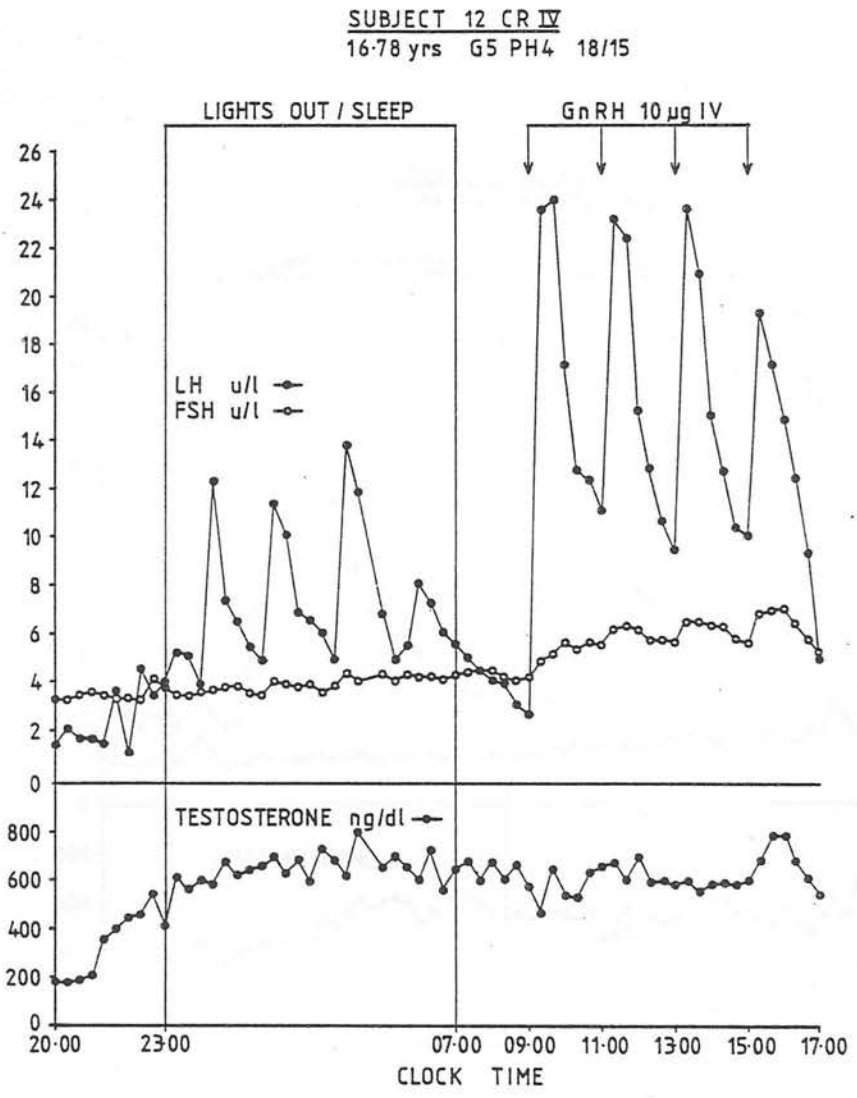

SUBJECT 11 CA I
15·66 yrs G2 PH1 5/5
LIGHTS OUT / SLEEP
GnRH 10 µg IV
LH u/l
FSH u/l
TESTOSTERONE ng/dl
14
12
10
8
6
4
2
0
800
600
400
200
0
20·00
23·00
07·00
09·00
11·00
13·00
15·00
17·00
CLOCK TIME

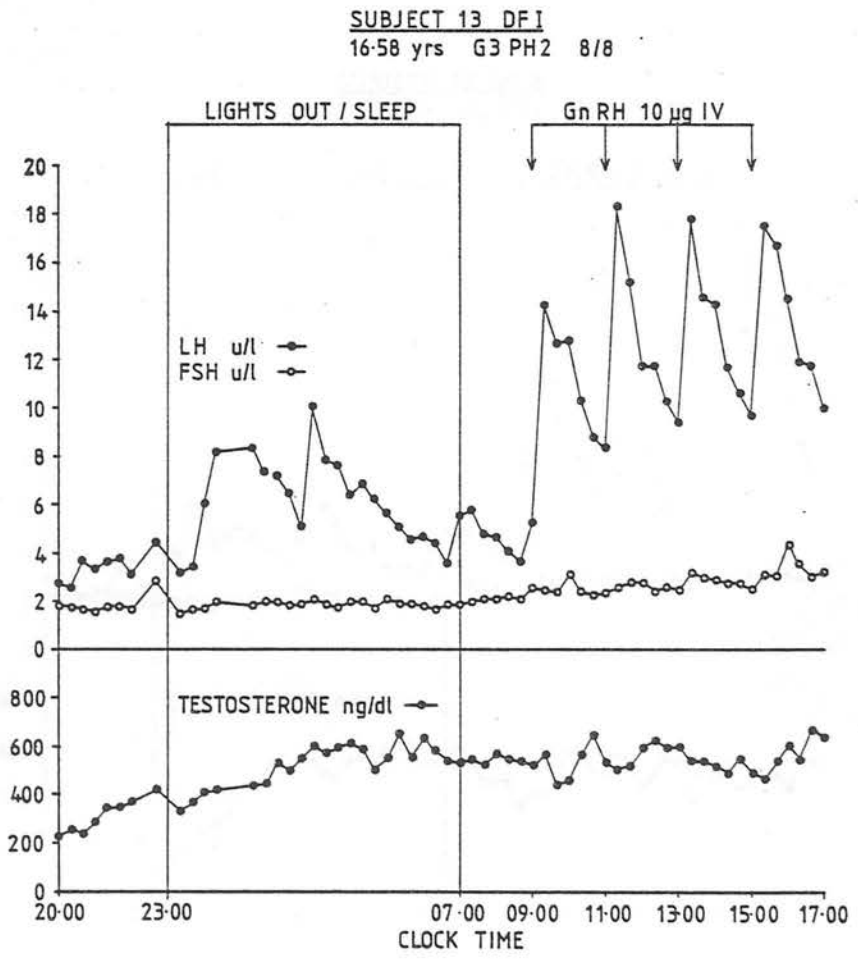

SUBJECT 11 CA II
16·53 yrs G3 PH2 10/10
LIGHTS OUT / SLEEP
Gn RH 10 µg IV
LH u/l
FSH u/l
TESTOSTERONE ng/dl
22
20
18
16
14
12
10
8
6
4
2
0
800
600
400
200
0
20·00
23·00
07·00
09·00
11·00
13·00
15·00
17·00
CLOCK TIME

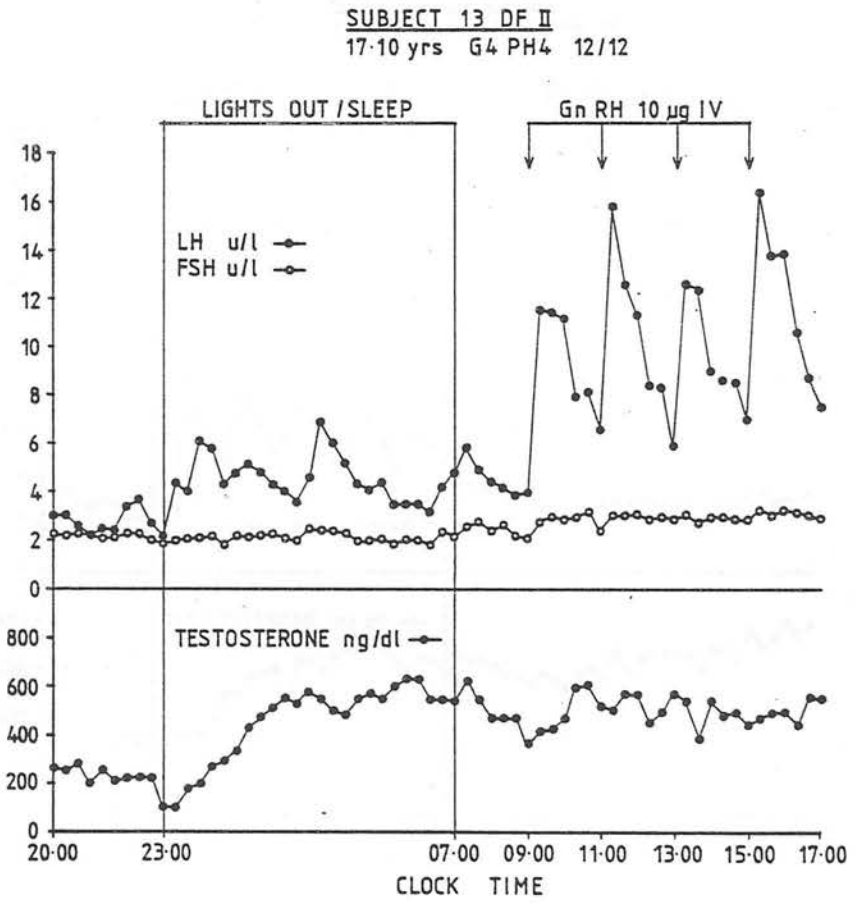

SUBJECT 12 CR I
14·58 yrs G2 PH2 5/5
LIGHTS OUT / SLEEP
Gn RH 10 µg IV
LH u/l
FSH u/l
TESTOSTERONE ng/dl
18
16
14
12
10
8
6
4
2
0
800
600
400
200
0
20·00
23·00
07·00
09·00
11·00
13·00
15·00
17·00
CLOCK TIME

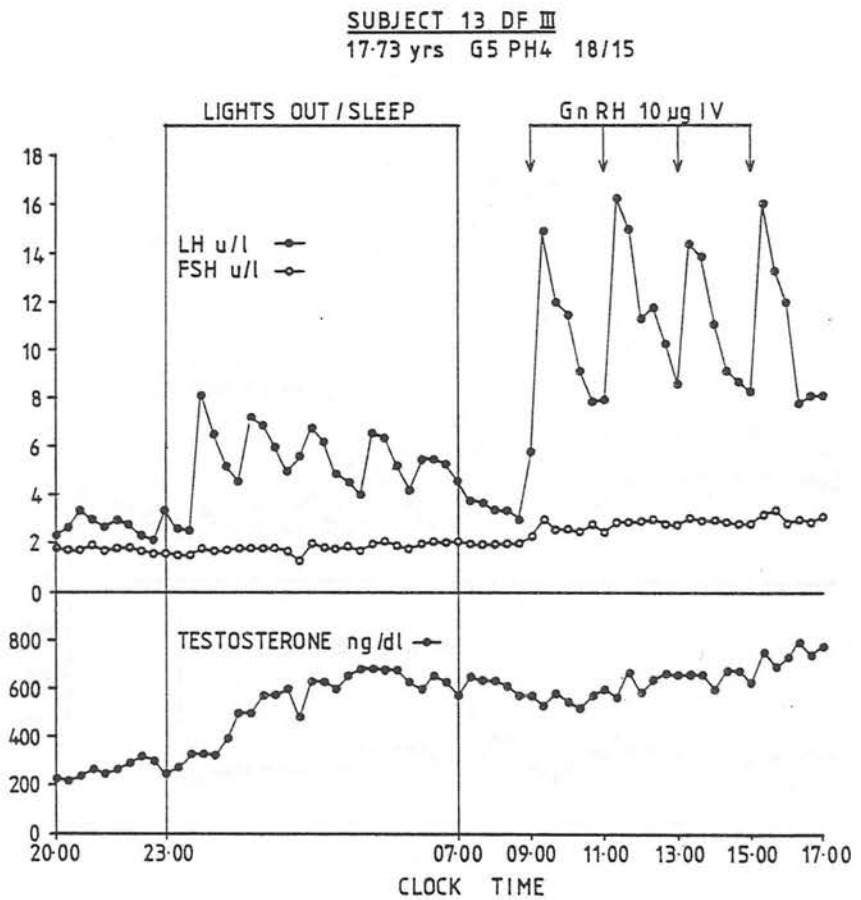

SUBJECT 12 CR II
15·10 yrs G3 PH3 8/8
LIGHTS OUT / SLEEP
GnRH 10 µg IV
LH u/l
FSH u/l
TESTOSTERONE ng/dl
22
20
18
16
14
12
10
8
6
4
2
0
800
600
400
200
0
20·00
23·00
07·00
09·00
11·00
13·00
15·00
17·00
CLOCK TIME

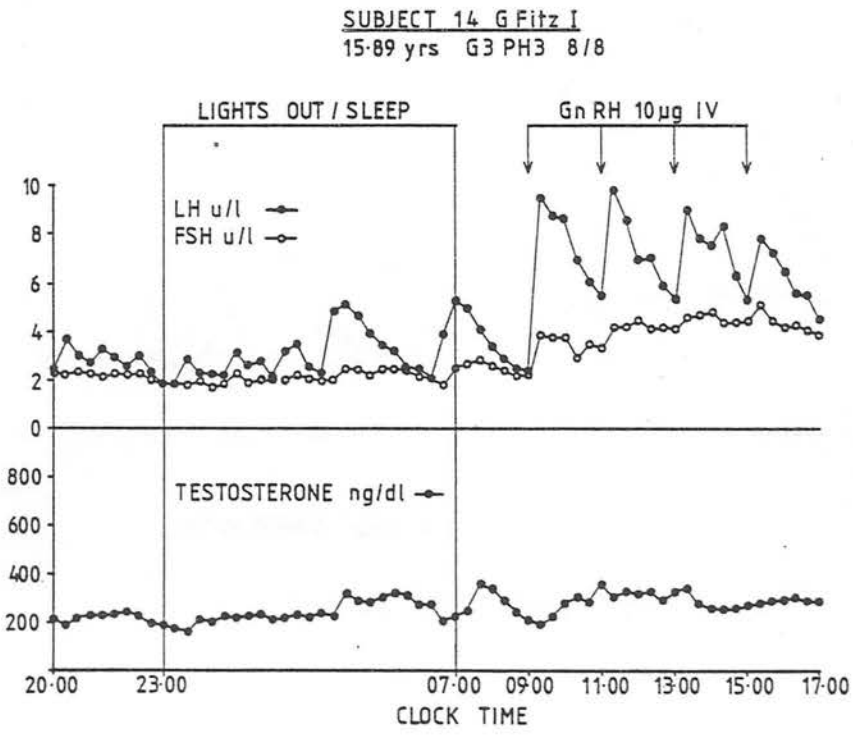

SUBJECT 12 CR III
15·58 yrs G4 PH3 10/10
LIGHTS OUT / SLEEP
Gn RH 10 µg IV
LH u/l
FSH u/l
TESTOSTERONE ng/dl
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
800
600
400
200
0
20·00
23·00
07·00
09·00
11·00
13·00
15·00
17·00
CLOCK TIME

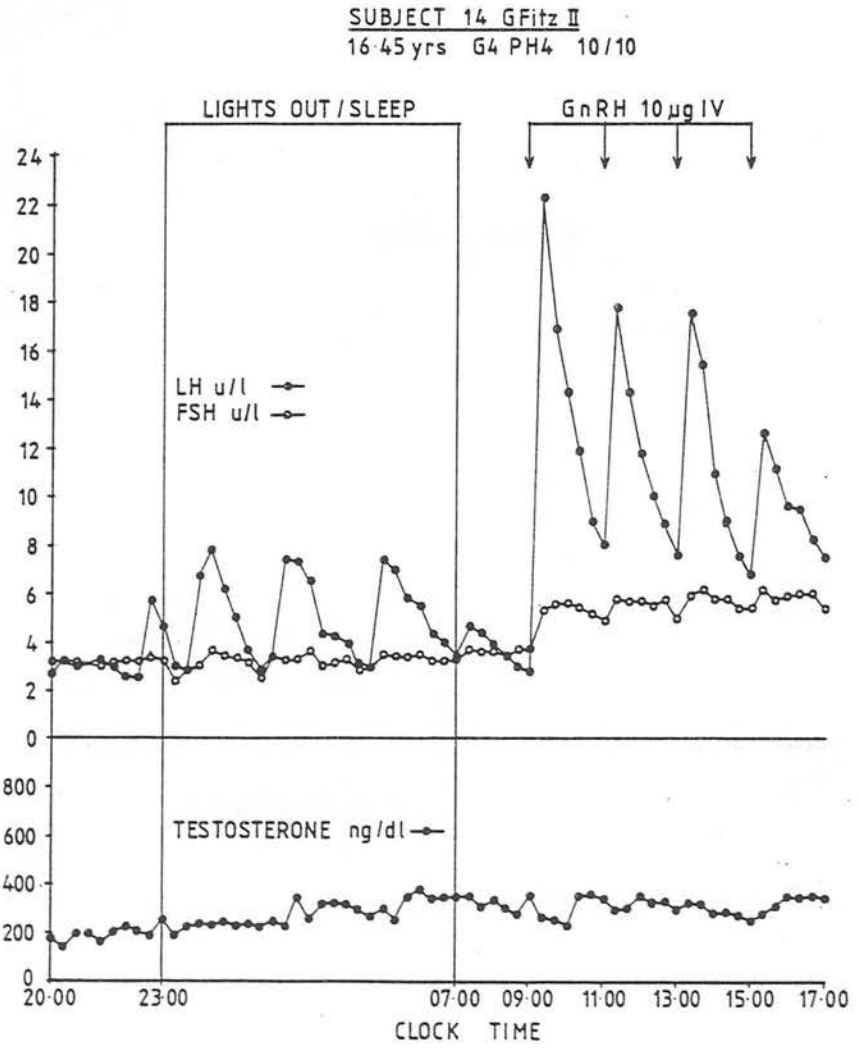

SUBJECT 12 CR IV
16·78 yrs G5 PH4 18/15
LIGHTS OUT / SLEEP
GnRH 10 µg IV
LH u/l
FSH u/l
TESTOSTERONE ng/dl
26
24
22
20
18
16
14
12
10
8
6
4
2
0
800
600
400
200
0
20·00
23·00
07·00
09·00
11·00
13·00
15·00
17·00
CLOCK TIME

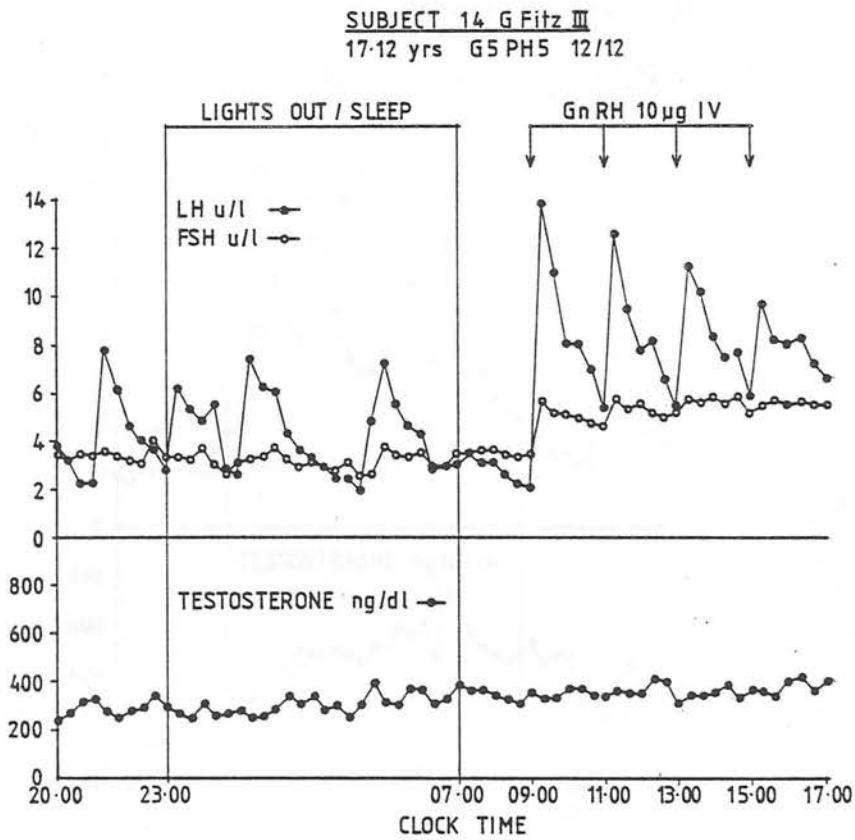

SUBJECT 13 DFI
16·58 yrs G3 PH2 8/8
LIGHTS OUT / SLEEP
Gn RH 10 µg IV
LH u/l
FSH u/l
TESTOSTERONE ng/dl
20·00
23·00
07·00
09·00
11·00
13·00
15·00
17·00
CLOCK TIME

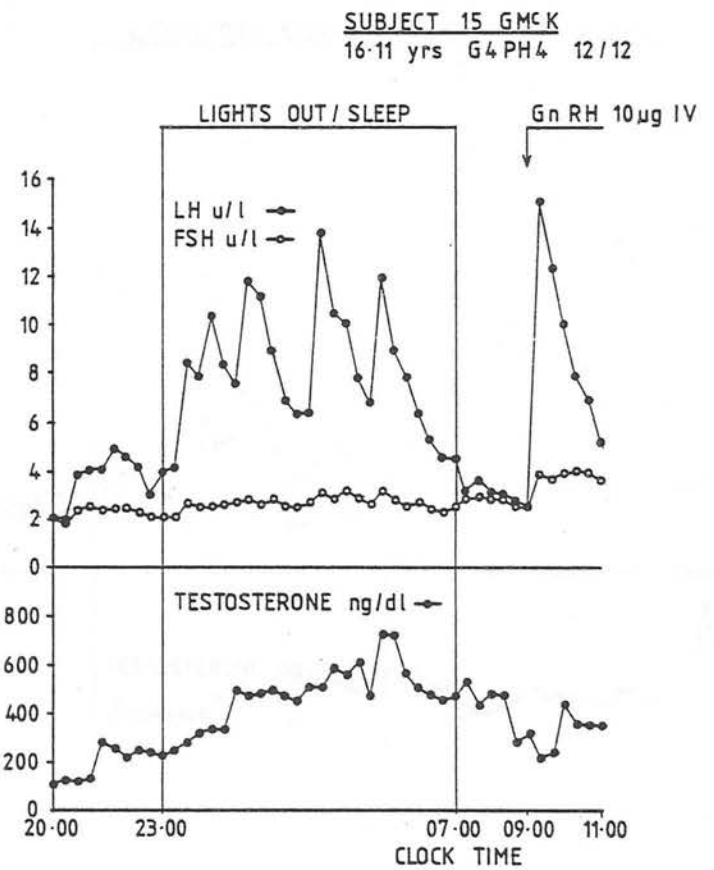

SUBJECT 13 DF II
17·10 yrs G4 PH4 12/12
LIGHTS OUT / SLEEP
Gn RH 10 µg IV
LH u/l
FSH u/l
TESTOSTERONE ng/dl
18
16
14
12
10
8
6
4
2
0
800
600
400
200
0
20·00
23·00
07·00
09·00
11·00
13·00
15·00
17·00
CLOCK TIME

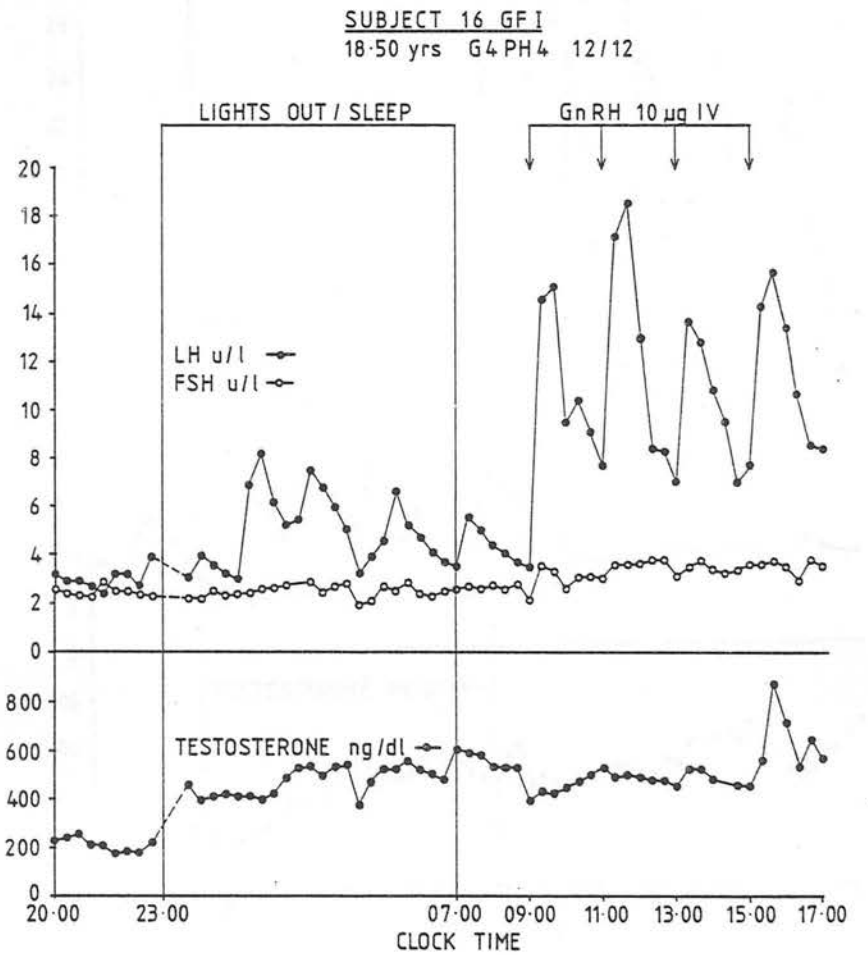

SUBJECT 13 DF III
17·73 yrs G5 PH4 18/15
LIGHTS OUT / SLEEP
Gn RH 10 µg I V
LH u/l
FSH u/l
TESTOSTERONE ng/dl
18
16
14
12
10
8
6
4
2
0
800
600
400
200
0
20·00
23·00
07·00
09·00
11·00
13·00
15·00
17·00
CLOCK TIME

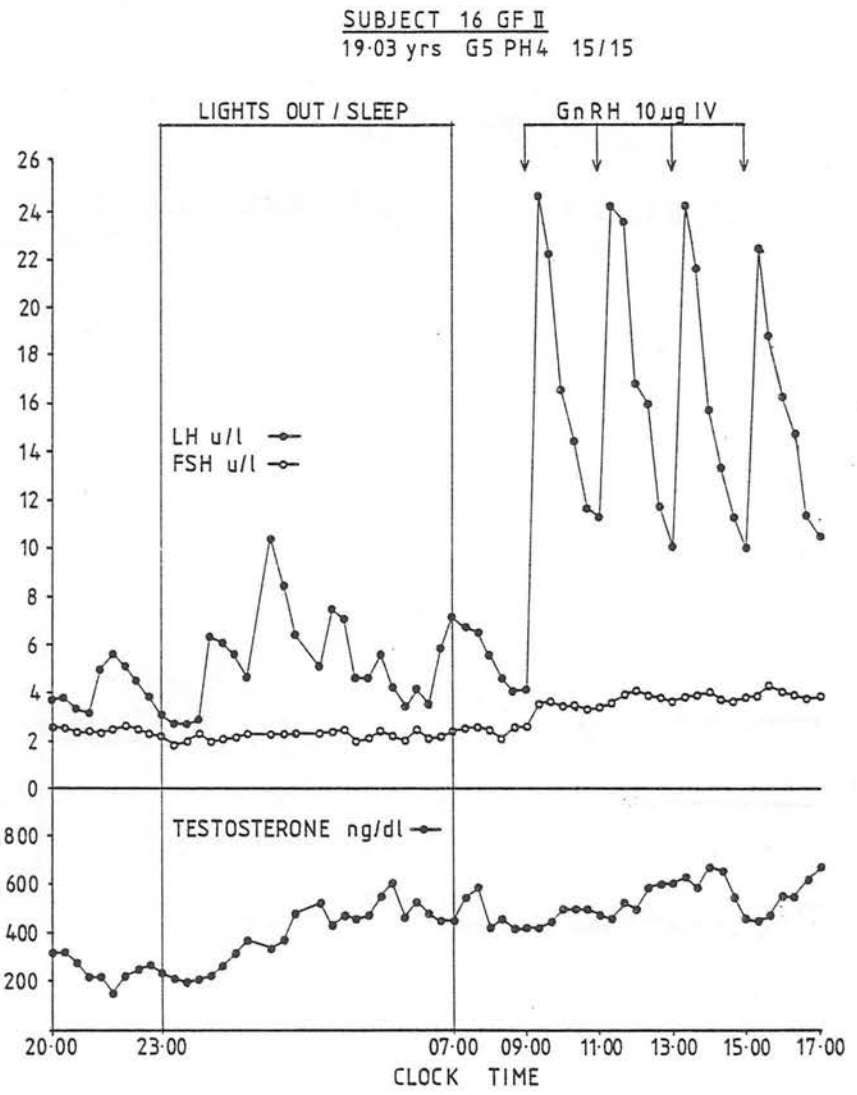

SUBJECT 14 G Fitz I
15·89 yrs G3 PH3 8/8
LIGHTS OUT / SLEEP
Gn RH 10µg IV
LH u/l
FSH u/l
TESTOSTERONE ng/dl
10
8
6
4
2
0
800
600
400
200
20·00
23·00
07·00
09·00
11·00
13·00
15·00
17·00
CLOCK TIME

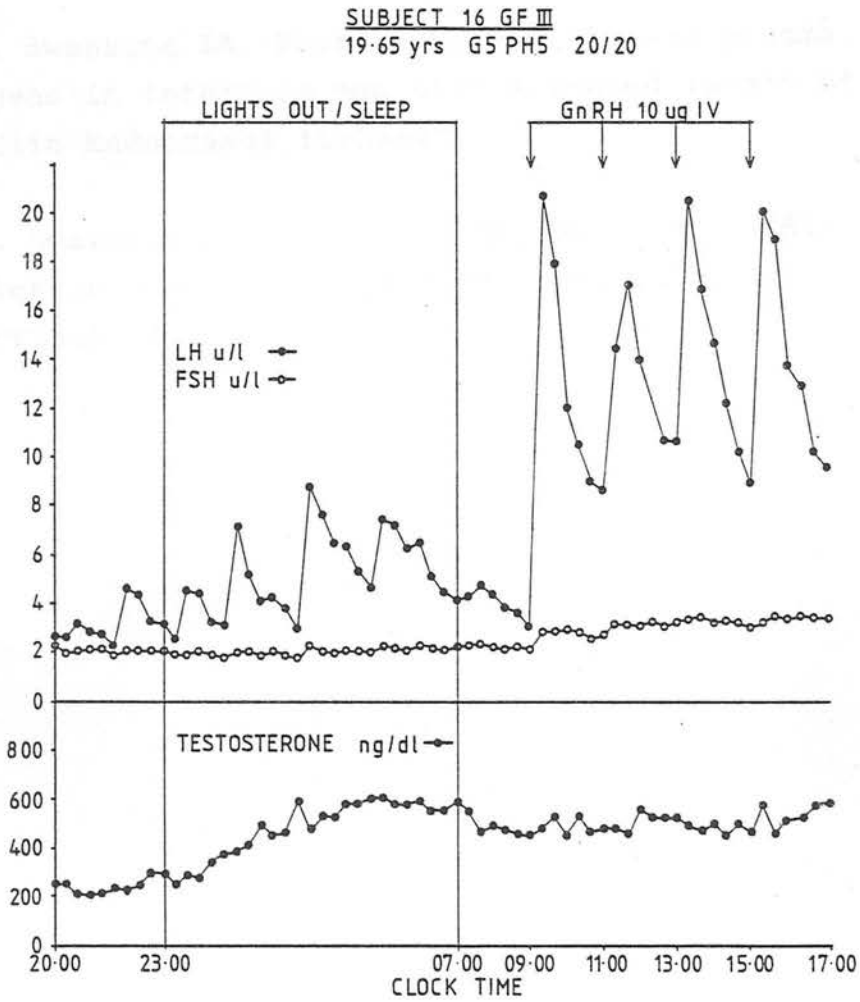

SUBJECT 14 GFitz II
16·45 yrs G4 PH4 10/10
LIGHTS OUT / SLEEP
GnRH 10 µg IV
LH u/l
FSH u/l
TESTOSTERONE ng/dl
24
22
20
18
16
14
12
10
8
6
4
2
0
800
600
400
200
0
20·00
23·00
07·00
09·00
11·00
13·00
15·00
17·00
CLOCK TIME


SUBJECT 14 G Fitz III
17·12 yrs G5 PH5 12/12
LIGHTS OUT / SLEEP
Gn RH 10µg IV
LH u/l
FSH u/l
TESTOSTERONE ng/dl
14
12
10
8
6
4
2
0
800
600
400
200
0
20·00
23·00
07·00
09·00
11·00
13·00
15·00
17·00
CLOCK TIME


SUBJECT 15 GMcK
16·11 yrs G4PH4 12/12
LIGHTS OUT / SLEEP
Gn RH 10µg IV
LH u/l
FSH u/l
TESTOSTERONE ng/dl
16
14
12
10
8
6
4
2
0
800
600
400
200
0
20·00
23·00
07·00
09·00
11·00
CLOCK TIME


SUBJECT 16 GF I
18·50 yrs G4 PH4 12/12
LIGHTS OUT / SLEEP
GnRH 10 µg IV
LH u/l
FSH u/l
TESTOSTERONE ng/dl
20·00
23·00
07·00
09·00
11·00
13·00
15·00
17·00
CLOCK TIME


SUBJECT 16 GF II
19·03 yrs G5 PH4 15/15
LIGHTS OUT / SLEEP
GnRH 10 µg IV
LH u/l
FSH u/l
TESTOSTERONE ng/dl
26
24
22
20
18
16
14
12
10
8
6
4
2
0
800
600
400
200
20·00
23·00
07·00
09·00
11·00
13·00
15·00
17·00
CLOCK TIME


SUBJECT 16 GF III
19·65 yrs G5 PH5 20/20
LIGHTS OUT / SLEEP
GnRH 10 ug IV
LH u/l
FSH u/l
TESTOSTERONE ng/dl
20·00
23·00
07·00
09·00
11·00
13·00
15·00
17·00
CLOCK TIME

APPENDIX III

The studies in Section C have been published in the following papers.

1 Wu FCW, Edmond P, Raab G, Hunter WM (1981) Endocrine assessment of the subfertile male. Clin Endocrinol 14:493-507

2 Wu FCW, Swanston IA, Baird DT (1982) Raised plasma oestrogens in infertile men with elevated levels of FSH. Clin Endocrinol 16:39-47.

3 Wu FCW, Swanston IA, Hargreave TB, Baird DT (1982) Human testis does not secrete oestrone sulphate. J Endocrinol 92:185-194.

Clinical Endocrinology (1981) **14**, 493–507

ENDOCRINE ASSESSMENT OF THE SUBFERTILE MALE

F. C. W. WU, P. EDMOND, G. RAAB AND W. M. HUNTER

Departments of Obstetrics and Gynaecology, and Urology, University of Edinburgh, Medical Computing and Statistics Group, University of Edinburgh, MRC Immunoassay Team, Edinburgh

(Receieved 13 June 1980; revised 20 October 1980; accepted 29 November 1980)

SUMMARY

Sixty-three male (XY) patients attending a subfertility clinic with average sperm density under 40 million/ml were studied by testicular biopsy and multiple basal estimations of plasma LH, FSH, testosterone as well as LHRH (50 μg i.v.) stimulation. A further forty patients with similar sperm densities also had testicular biopsy but only single estimations of the three hormones. A single basal FSH was found to be the best discriminator of testicular histologies. Patients with testicular biopsies showing germ cell aplasia in some or all seminiferous tubules (grades 3 and 4) had significantly higher basal FSH than those with hypospermatogenesis, germ cell arrest or normal appearance (grades 1 and 2). Basal FSH also showed a linear trend rising with decreasing sperm density but only rose above the normal range when sperm densities fell below 1 million/ml. When basal FSH, testicular histology and sperm density were considered together in the whole group ($n = 100$), high levels of FSH accurately indicated the presence of germ cell aplasia in some or all seminiferous tubules in azoo- and oligospermic men with sperm density under 5 million/ml. Normal FSH and azoospermia is diagnostic of obstruction in the excurrent ducts, and further investigation is undertaken if surgical correction of the obstruction is contemplated. Hormone estimations are not helpful in oligospermic patients with average sperm density over 5 million/ml. On the basis of these findings it is suggested that there is little place for the LHRH test in the routine assessment of male subfertility. Testicular biopsy is indicated only in oligospermic patients with average sperm density under 5 million/ml and normal basal FSH.

Hormonal measurement, especially that of FSH, has recently added a new demension to the investigation of male infertility which has traditionally relied on semen analysis (Eliasson, 1975) and testicular biopsy (Charny, 1940). The finding of a reciprocal relationship between urinary or plasma FSH and the number of germ cells in seminiferous tubule cross-sections (Johnsen, 1970; Franchimont *et al.*, 1972; de Kretser *et al.*, 1974) has

Correspondence: F. C. W. Wu, Royal Infirmary, Lauriston Place, Edinburgh EH3 9YW.

0300-0664/81/0500-0493$02.00

provided a convenient quantitative measurement of spermatogenesis which could replace the invasive procedure of testicular biopsy. Sperm density has also been shown to have an inverse relationship to FSH (Rosen & Weintraub, 1971; Mauss & Börsch 1973; Kjessler & Wide, 1973; Hunter *et al.*, 1974; Christiansen, 1975; Aafjes *et al.*, 1977) although this was not confirmed by others (Franchimont *et al.*, 1972; de Kretser *et al.*, 1972; Leonard *et al.*, 1972). We therefore decided to evaluate further the efficacy of plasma FSH in assessiing the degree of seminiferous tubular damage at different levels of sperm density in men presenting with infertility.

Exaggerated FSH response to LHRH proportional to basal levels has been reported in patients with oligospermia and azoospermia (Isurgi *et al.*, 1973; Mecklenberg & Sherins, 1974; Franchimont *et al.*, 1975; Roulier *et al.*, 1976; Guay *et al.*, 1977). However, Lipschultz *et al.* (1977) found exaggerated FSH response to LHRH in oligospermic patients with normal basal FSH levels, implying that the LHRH response (rather than basal FSH) may be a more sensitive index of seminiferous tubular function. The clinical value of the LHRH test, in comparison with basal FSH measurements in male subfertility, therefore needs further evaluation.

The presence of abnormal Leydig cell function in subfertile males with more severe forms of seminiferous tubular damage was indicated by elevated basal LH (Kjessler & Wider, 1973; Mauss & Börsch 1973; Christiansen, 1975; Hunter *et al.*, 1974), exaggerated LH response to LHRH stimulation (Isurgi *et al.*, 1973; Mecklenberg & Sherins, 1974; de Kretser *et al.*, 1975a; Roulier *et al.*, 1976; Guay *et al.*, 1977) and more directly by statistically lower testosterone levels and impaired response to hCG stimulation (de Kretser *et al.*, 1972; 1975b). Study of the LH-testosterone axis may therefore also furnish diagnostically useful information in addition to FSH measurements in the investigation of male fertility status.

Since LH and testosterone, and to a lesser extent FSH, are secreted episodically (Nankin & Troen, 1971; Naftolin *et al.*, 1972; Santen & Bardin, 1973; Alford *et al.*, 1973; Smith *et al.*, 1974) multiple measurements of these hormones may provide more accurate information than single estimations.

The aims of the present study were to evaluate and compare the clinical application of: (1) single basal measurements of FSH, LH and testosterone; (2) multiple measurements of FSH, LH and testosterone and (3) the LHRH response, in the assessment of subfertile males in terms of their relationship with testicular histology and sperm production. Having established the best hormone parameter(s) and their limitations the indications for testicular biopsy can then be rationalized.

PATIENTS AND METHODS

Patients

A total of 103 consecutive patients referred to the Male Subfertility Clinic, Royal Infirmary, Edinburgh with average sperm density under 40 million/ml were studied. All patients were normally virilized and had normal male chromosome karyotype (46XY). The median age was 34 years and range 24–45 years. The clinical histories, summarized in Table 1, revealed no abnormalities in three-quarters of the group. A history of unilateral or bilateral cryptorchidism could be elicited in eleven patients while six had infections in the genital tract. One patients received radiotherapy to the spine for ankylosing spondylitis while another underwent unilateral orchidectomy following a road accident.

Table 1. Summary of clinical findings in 103 patients attending a subfertility clinic with average sperm density under 40 million/ml

History		Physical findings	
Nil of note	82	Normal external genitalia	51
Unilateral undescended or retractile testis	6	Bilateral small testes	27
Bilateral undescended or retractile testes	5	Unilateral small testis	7
Genital infections	6	Varicocoele	12
Irradiation	1	Epididymal or vas abnormalities	4
Trauma	1	Phimosis	1
Sexual difficulties	2	Hydrocoele	1

Two patients reported psychosexual difficulties. No abnormalities were found on physical examination in half of the patients (Table 1). Bilateral (twenty-seven) and unilateral (seven) small testes–under 15 ml volume as measured by Prader's orchidometer, were found in thirty-four patients. Twelve patients had clinically detectable varicocoeles and four had palpable abnormalities of the epididymis or vas deferens. Phimosis and hydrocoele were each present in one patient.

Thirteen healthy normal subjects who had fathered one or more children within the past 5 years were also investigated hormonally as controls. Their median age was 31 years and range 24–37 years.

Endocrine investigations

In the first sixty-three patients and the thirteen controls, basal samples of LH, FSH and testosterone were obtained at 30 min intervals for $1\frac{1}{2}$ h. This was followed by an i.v. bolus injection of 50 μg LHRH (Gonadorelin, Ayerst) and samples for LH and FSH collected at 30, 60 and 90 min afterwards. In the subsequent forty patients, only single samples of basal LH, FSH and testosterone were obtained.

Hormone assays

Gonadotrophins were measured by a double-antibody radioimmunoassay employing the LH standard 68/40 (77 u/ampoule) and FSH standard 69/104 (10 u/ampoule) from the National Institute of Biological Standards and control, London (Hunter & Bennie, 1978). Within assay coefficients of variation for LH and FSH were 8·0 and 7·0% respectively. Interassay and intra-assay coefficients of variation were 8·6 and 7·4% for LH and FSH respectvely. The limit of detection, arbitrarily set at 15% inhibition of Bo (corresponding to 6 × SD of Bo), was 0·56 u/l for LH and 0·74 u/l for FSH. The normal range was determined in 100 healthy adult male blood donors aged 18–49 and the normal limits set at the fifth to ninety-fifth percentile for LH (3·3–10·0 u/l) and FSH (1·7–6·7 u/l).

Plasma testosterone was measured by radioimmunoassay without chromatography (Corker & Davidson, 1978). The anti-testosterone-3-carboxymethyloxime-ovalbumen antiserum (HP/S55-IIA Guilhday) had 32% cross reaction with 5α-dihydrotestosterone and 21% with 4-androstenediol. Separation of bound from unbound tracer was effected by dextran-charcoal suspension. The intra-assay and interassay coefficients of variation

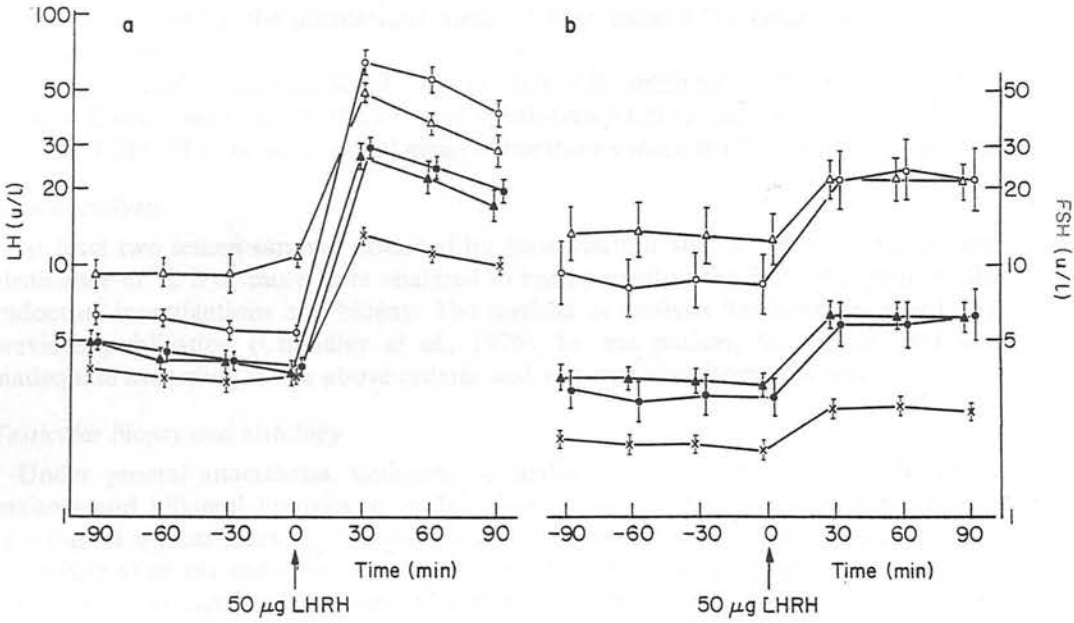


Fig. 1. Profiles of mean basal gonadotrophins (**a**LH **b**FSH) and response to 50 μg of LHRH i.v. in the four grades of testicular histology (see text) in sixty-one patients and thirteen normal controls. Biopsy 1 ●; biopsy 2 ▲; biopsy 3 ○; biopsy 4 △; control x.

were 6·7 and 10·8% respectively. Pooled equal aliquots from the four testosterone samples from each patient were assayed. The normal range for adult males (10–35 nmol/l) was determined in fifty-six fertile men aged 24–50 years with at least one child under the age of two, who requested vasectomy.

Analysis of LHRH response

Examination of the LH and FSH profiles after LHRH stimulation (Fig. 1) suggested that a model of exponential decline might be appropriate, with a relatively slower decline in FSH which might effectively be zero for the duration of the test (i.e. constant stimulated level). All gonadotrophin values were log transformed since both LH and FSH in the 100 blood donors had log normal distributions. For each individual, a straight line fit to log (stimulated-mean basal) LH and FSH values with time was obtained by least squares. Pooled estimates of the slopes of these straight lines for the whole group were obtained for each gonadotrophin. This assumed that the half-life for the hormones were the same for all subjects studied. This assumption was tested by analysis of variance between individual and common slope models, and confirmed the linear decrease in log (peak-mean basal) LH with a common slope for all subjects which corresponded to a common half-life of 1·36 h (1·20–1·56—95% confidence interval) after LHRH stimulation. It also confirmed a constant stimulated level of FSH for each individual, with no increase or decrease between 30 and 90 min after LHRH stimulation. In the following analyses, the gonadotrophin measures for each individual were designated as follows:

1 Basal LH (LB): the geometrical mean of four basal LH values before LHRH stimulation.

2 Basal FSH (FB): the geometrical mean of four basal FSH values before LHRH stimulation.
3 Peak LH (LP): estimated 30 min value (minus LB) obtained by fitting a straight line with the common slope to the set of log (peak-basal) LH readings for each individual.
4 Peak FSH (FP): the geometrical mean of the three stimulated FSH values minus FB.

Semen analysis

At least two semen samples obtained by masturbation after a period of ejaculatory abstinence of 72 h or more were analysed in each patient in the 3 months prior to the endocrine investigations and biopsy. The method of analysis has been described in a previous publication (Chandley *et al.*, 1976). In one patient, the semen data was inadequate according to the above criteria and was excluded from analysis.

Testicular biopsy and histology

Under general anaesthesia, unilateral testicular biopsies were performed in ninety patients and bilateral biopsies in twelve. One of the latter group had very different histological appearances in the two testes and one patient refused to undergo the procedure after his endocrine investigations. These two patients were excluded from subsequent analyses involving testicular histology. The biopsies were fixed in Bouin's solution and embedded in paraffin. The sections (5μ thick) were stained with haematoxylin and eosin. Testicular histology was graded according to the proportion of seminiferous tubules showing spermatogenic activity and the degree of such activity within tubules (McIlree *et al.*, 1966; Chandley *et al.*, 1976). Definition of the gradings were as follows:

Grade 1. All tubules show active spermatogenesis with production of mature sperms.
Grade 2. Some or all tubules show depression or arrest of spermatogenesis at various stages or a diminished number of germ cells. No tubules show a complete absence of germ cells.
Grade 3. Some but not all tubules show a complete absence of germ cells and contain only Sertoli cells.
Grade 4. Germ cells are absent from all tubules examined, their only content being Sertoli cells.

Thickening of the tunica propria, hyalinization of the tubules, or increase in Leydig cell numbers may occur in grades 3 and 4.

RESULTS

The distribution of testicular histology and sperm density in the 100 patients is presented in Table 2. Of the twenty-four patients with grade 3 and 4 histology only one had an average sperm density over 5 million/ml. In contrast, the full range of sperm density from azoospermia to 40 million/ml was encountered in the seventy-six patients with grade 1 and 2 histology. In patients with sperm density over 5 million/ml, all except one had grade 1 or 2 histology. In those with sperm density under 5 million/ml, any histological grade could be present. It is in this last group that hormone studies may prove to be most helpful.

The relationship between testicular histology and the gonadotrophin and testosterone levels in the first sixty-three patients who underwent LHRH testing is shown in Table 3.

Table 2. The distribution of sperm density and testicular histology in 100 patients. Three patients had been excluded due to inadequate information in semen analyses (1) and testicular histology (2).

Sperm density	Azoo.	⩽1·0 M/ml	1·1–5·0 M/ml	5·1–10·0 M/ml	10·1–20·0 M/ml	20·1–40·0 M/ml	Total
Testicular Histology							
Grade 1	11	2	11	8	15	7	54
Grade 2	2	2	6	4	4	4	22
Grade 3	1	11	2	0	1	0	15
Grade 4	5	3	1	0	0	0	9
Total	19	18	20	12	20	11	100

Although there was no significant difference in testosterone between any of the patient or control groups, the four gonadotrophin measures (FB, FP, LB & LP) separated the patients into broad histological groups: grade 1 with 2 and grade 3 with 4. This pooling of gonadotrophin measures in terms of similar means and standard deviations is also illustrated by the profiles of responses to LHRH in Fig. 1.

To determine the best gonadotrophin measure(s) to distinguish between the two histological groupings (grades 1 and 2 from grades 3 and 4), multivariate discriminant analysis (Marriot, 1974) was used. This calculates the generalized distances (Mahalanobis's D) which give the differences between the two patient groups and controls in standard deviation units allowing for the correlation between the variables (Table 4). Either basal FSH or peak FSH alone could provide the best separation between the two histological groupings and no increase in discrimination was obtained by including further hormonal parameters. The difference in LH between the histological groups could entirely be explained by the correlation between LH and FSH measures. Basal and peak FSH were highly correlated ($r=0{\cdot}75$, $P<0{\cdot}0001$) in the various patient and control groups (Fig. 2), so that either one of these measures would not improve the discriminant function of the other. Using basal FSH alone with the upper limit of the normal range at 6·7 u/l, elevated values in 81% (thirteen of sixteen) of grade 3 and 4 biopsies and normal values in 91% (forty-one of forty-five) of grade 1 and 2 biopsies were found.

The best discrimination between normal controls and the two subfertile patient groups was provided by the LH measures (Table 4). Peak LH was the best single parameter distinguishing controls from patients with grade 1 or 2 histology. The addition of other measures did not significantly improve the discrimination. Although either peak FSH or peak LH alone provided a very clear cut separation between controls and patients with grade 3 or 4 histology, the best discrimination was given by the combination of basal and peak LH.

The relationship between sperm density and the five hormonal parameters is shown in Table 5. Five azoospermic patients with normal FSH and grade 1 or 2 testicular histologies were considered to have obstructions in the excurrent ducts and were excluded from this analysis. Both basal and peak FSH showed a significant linear rising trend ($P<0{\cdot}0001$) with decreasing sperm density (Fig. 3). Mean basal FSH remained fairly constant until sperm densities fell below 5 million/ml and only exceeded the upper limit of normal basal

Table 3. Mean levels and 95% confidence limit in parenthesis of basal and peak (response to 50 μg LHRH i.v.) gonadotrophins and basal testosterone in the four grades of testicular histology (see text) and controls. Patients with testicular histology grade 1 were pooled with grade 2 and grade 3 with grade 4 since the hormone levels were not significantly different in these subgroups

Testicular histology	Number of patients	Basal FSH (u/l)	Peak FSH (u/l)	Basal LH (u/l)	Peak LH (u/l)	Testosterone (nmol/l)
Grade 1	33	3·32 (2·69–4·10)	2·10 (1·49–2·94)	4·39 (3·74–5·15)	18·73 (14·88–23·57)	21·75 (19·87–23·63)
Grade 2	12	2·53 (1·80–3·56)	2·29 (1·30–4·06)	4·14 (3·32–5·16)	16·78 (11·94–23·57)	20·34 (17·62–23·06)
Grade 3	9	9·30 (6·11–14·15)	13·07 (8·17–20·91)	5·42 (4·62–6·36)	39·25 (30·27–50·91)	21·66 (16·88–26·64)
Grade 4	7	13·20 (8·76–19·89)	7·61 (5·58–10·38)	9·58 (7·54–12·18)	25·79 (19·30–34·47)	21·06 (17·11–25·01)
Grade 1 and 2	45	3·10 (2·66–3·61)	2·14 (1·67–2·24)	4·35 (3·89–4·87)	18·17 (15·41–21·44)	21·04 (19·71–22·37)
Grade 3 and 4	16	10·80 (8·09–14·43)	10·28 (7·73–13·67)	6·96 (5·84–8·29)	32·79 (27·04–39·76)	21·36 (18·51–24·21)
Control	13	1·86 (1·59–2·18)	0·61 (0·42–0·90)	3·53 (3·04–4·09)	9·78 (8·51–11·23)	20·18 (17·46–22·94)

Table 4. Discriminant functions of the hormone (see text) basal LH (LB), basal FSH (FB), peak LH (LP) and peak FSH (FP) in differentiating testicular histological groups and controls as represented by the generalized distance (D) between them

	Generalized distance (D)		
Hormone measure	Testicular histology grades 1 & 2 v. 3 and 4	Testicular histology grades 1 and 2 v. controls	Testicular histology grades 3 and 4 v. controls
LB alone	1·07	0·22	1·88
FB alone	2·00	0·50	3·31
LP alone	0·97	1·46	3·89
FP alone	1·71	1·34	4·31
Best pair	FB + FP 2·18	LP + FP 1·58	LB + LP 5·36
All four together	2·22	1·60	5·85

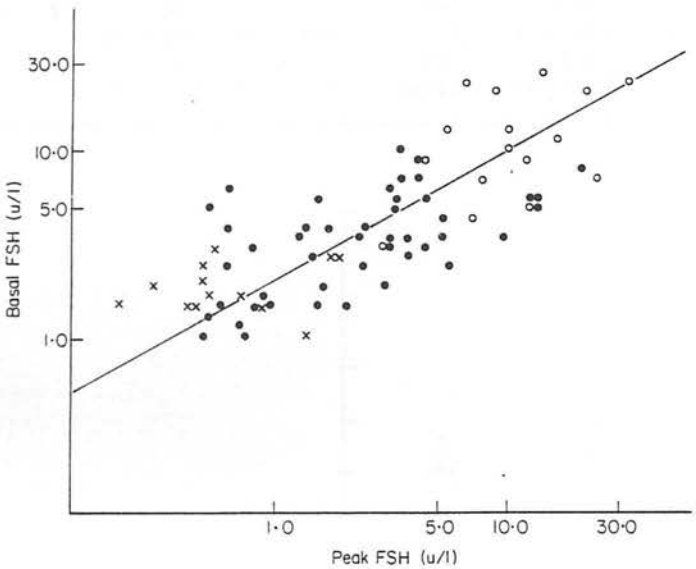


Fig. 2. Basal and peak FSH (response to 50 μg LHRH i.v.) were highly correlated in the sixty-one patients and thirteen controls. Coefficient of correlation r = 0·749 and $P < 0{\cdot}00001$. Biopsy 1 & 2 ($n = 45$) •; biopsy 3 & 4 ($n = 16$) ○; normal x.

FSH range (6·7 u/l) with sperm densities under 1 million/ml. Peak FSH showed a linear rising trend over the whole range of sperm densities from 40 million/ml to azoospermia, but the increase at each level was smaller than basal FSH. Neither testosterone basal LH or peak LH differed significantly with changes in sperm density, but the latter did show a weak degree of linearity.

Thus in the analysis of the first sixty-three patients, basal FSH was the best single

Table 5. Mean levels and 95% confidence limit in parenthesis of basal and peak (response to 50μg LHRH i.v.) gonadotrophins and basal testosterone at different sperm densities. Patients with obstructive azoospermia had grade 1 or 2 testicular histologies and major abnormalities in the efferent ducts

Sperm density	Number of patients	Basal FSH (u/l)	Peak FSH (u/l)	Basal LH (u/l)	Peak LH (u/l)	Testosterone (nmol/l)
Non-obstructive azoospermia	3	17·82 (6·38–49·83)	7·39 (2·45–22·08)	10·63 (10·11–11·18)	22·12 (9·67–50·61)	22·86 (17·55–28·17)
⩽1·0 M/ml	13	8·80 (6·07–12·76)	9·96 (6·73–14·73)	5·68 (4·50–7·17)	32·11 (24·88–41·43)	19·61 (16·83–22·39)
1·1–5·0 M/ml	11	4·32 (3·44–5·41)	3·57 (2·00–6·38)	4·24 (3·11–5·76)	22·97 (14·90–35·41)	21·99 (19·37–24·61)
5·1–10·0 M/ml	6	2·69 (1·40–5·17)	2·83 (0·90–8·85)	4·93 (1·25–6·98)	20·81 (14·06–30·79)	20·53 (15·13–25·93)
10·1–20·0 M/l	16	3·18 (2·45–4·12)	2·33 (1·66–3·27)	4·56 (4·18–4·97)	19·69 (14·53–26·68)	22·81 (20·03–25·39)
20·1–40·0 M/l	8	3·40 (2·02–5·73)	1·22 (0·64–2·31)	4·65 (3·07–7·04)	13·46 (9·56–18·94)	19·11 (16·34–21·88)
Obstructive azoospermia	5	2·62 (1·50–4·60)	1·22 (0·59–2·49)	4·16 (2·45–7·05)	16·18 (11·67–22·43)	21·98 (14·79–29·17)
Normal controls	13	1·86 (1·59–2·18)	0·61 (0·42–0·90)	3·53 (3·04–4·09)	9·78 (8·51–11·23)	20·20 (17·46–22·94)

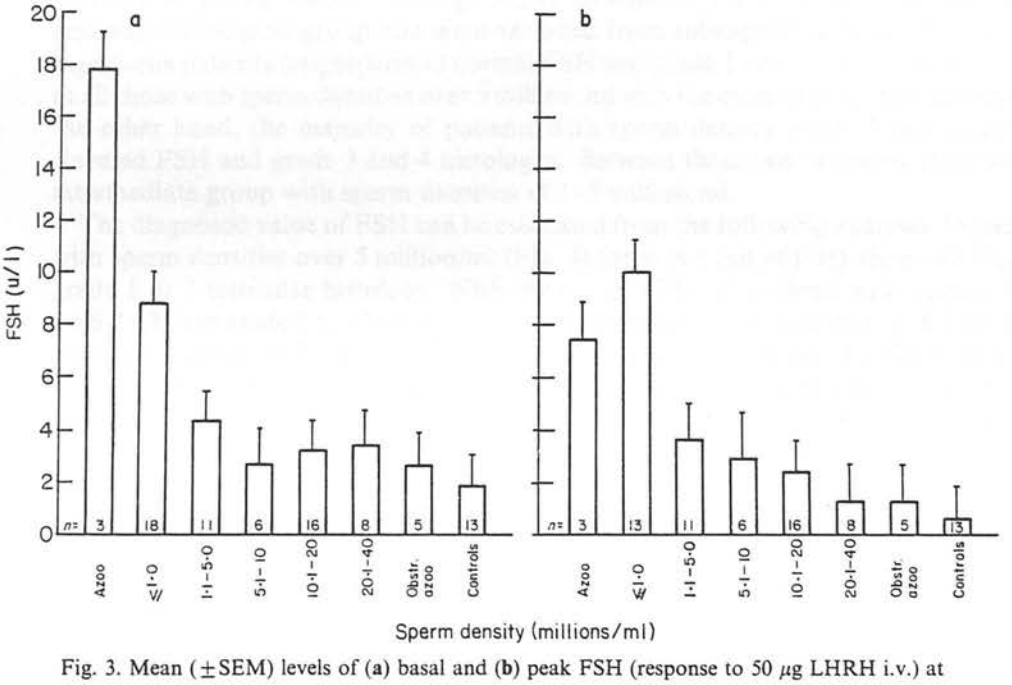


Fig. 3. Mean (±SEM) levels of (**a**) basal and (**b**) peak FSH (response to 50 μg LHRH i.v.) at different sperm densities. Patients with obstructive azoospermia had grade 1 or 2 histologies and major abnormalities of the efferent ducts.

hormonal parameter in terms of its relationship to testicular histology and sperm density. The LHRH response did not contribute any additional useful information in these respects. The within-patient coefficient of variation for four basal samples of FSH at 30 min intervals was so small (10%) that using a single measurement instead of the mean of four would not alter the above analyses significantly.

In the other forty patients, only single samples of basal FSH, basal LH, testosterone, and testicular histology were analysed. Discriminant analysis again confirmed basal FSH to be the best parameters in distinguishing the two histological categories. The addition of basal LH and testosterone did not improve the discriminant function. Elevated basal FSH was found in 75% (four of six) of grade 3 and 4 histologies and normal FSH in 97% (thirty of thirty-one) of grade 1 and 2 histologies. This was similar to the findings in the first sixty-three patients. The linear rising trend in basal FSH with decreasing sperm density was also confirmed. Thus the relationship between a single basal FSH and testicular histology and sperm density in the additional forty patients followed the same pattern in the first sixty-three.

The findings in 100 patients (three patients excluded from analysis due to insufficient data) are summarized in Fig. 4 and demonstrate the interrelationship between basal FSH, testicular histology and sperm density. In the nineteen azoospermic patients, basal FSH clearly differentiated the six with severe germ cell damage (grade 3 and 4 histologies) from the thirteen with grade 1 and 2 histologies. Of the latter thirteen patients, one had maturation arrest at the primary spermatocyte stage and five were confirmed to have bilateral agenesis of the vasa deferentia on scrotal exploration. One had patent vasa bilaterally on vasography and the obstruction was presumed to be in the epididymis. Six patients were not submitted to either scrotal exploration or vasography as they were reluctant to pursue further investigation or treatment. These thirteen patients form a separate aetiological group and were excluded from subsequent analyses. In the other eighty-one patients (oligospermic) normal FSH and grade 1 and 2 histologies were found in all those with sperm densities over 5 million/ml with the exception of three patients. On the other hand, the majority of patients with sperm density under 1 million/ml had elevated FSH and grade 3 and 4 histologies. Between these two extremes, there was an intermediate group with sperm densities of 1–5 million/ml.

The diagnostic value of FSH can be estimated from the following analyses. In patients with sperm densities over 5 million/ml (Fig. 4) forty-two out of forty-three (97·7%) had grade 1 or 2 testicular histology. With the use of FSH, all patients with normal levels ($< 6{\cdot}7$ u/l) had grade 1 or 2 histology. Of the three patients with elevated ($> 6{\cdot}7$ u/l) FSH, only one had grade 3 testicular histology. Thus the contribution of FSH is limited in patients with sperm densities over 5 million/ml who, with very few exceptions, are likely to have grade 1 or 2 histology. In patients with sperm density under 5 million/ml, 48% had grade 1 or 2 and 52% grade 3 or 4 histologies. With the use of FSH, 78% (eighteen of twenty-three) with normal values had grade 1 or 2 and 83% (eighteen of twenty-one) with elevated values had grade 3 or 4 histologies. In patients with sperm densities under 1 million/ml, 83% had grade 3 or 4 histology and only 17% had grade 1 or 2 histology. All patients with elevated FSH had grade 3 or 4 histology, but in those with normal FSH both histological groups occurred with similar frequencies—57% grades 3 and 4 and 42% grades 1 and 2. Thus if sperm density is under 5 or 1 million/ml, the finding of elevated FSH levels would confirm severe germ cell damage, but normal FSH is not helpful.

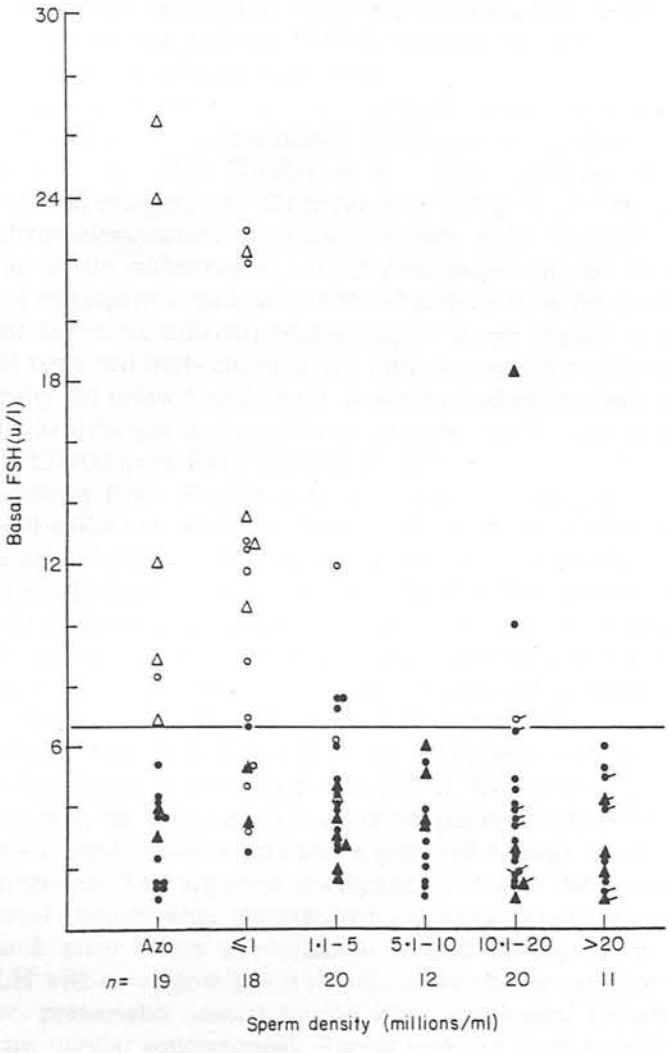


Fig. 4. Basal FSH, sperm density and testicular histology (grades 1–4—see text) in 100 subfertile patients. Horizontal line represents the upper limit of the normal range (6·7 u/l) for basal FSH in 100 healthy male blood donors. Markers indicate patients who subsequently impregnated their wives with or without treatment. Biopsy 1 •; biopsy 2 ▲; biopsy 3 ○; biopsy 4 △.

DISCUSSION

Our findings showed that in terms of the inverse relationships with sperm density and the degree of germ cell loss demonstrated by testicular histology, basal FSH, amongst the various combinations of hormonal parameters, gave the greatest discriminant value. This confirmed the findings in previous studies (Johnsen, 1970; Rosen & Weintraub, 1972; Kjessler & Wide, 1973; Mauss & Börsch, 1973; de Kretser *et al.*, 1974; Hunter *et al.*, 1974; Christiansen, 1975; Aafjes *et al.*, 1977). The variation of basal FSH at intervals of 30 min

was small and single estimations were representative. The additional measurements of basal LH, testosterone and the LHRH response did not contribute further to the discriminant function of basal FSH alone.

The finding that the FSH response to LHRH stimulation was highly correlated with basal FSH confirms the previous studies (Isurgi *et al.*, 1973; Mecklenberg & Sherins, 1974; Franchimont *et al.*, 1975; Roulier *et al.*, 1976). Lipschulz *et al.* (1977) however, demonstrated an exaggerated FSH response to 250 μg of LHRH in spite of normal basal levels in oligospermic patients with sperm density under 10 million/ml. This discrepancy may be due to the difference in LHRH dose employed and the different criteria for selection of oligospermic patients (under 40 million/ml in the present study). A further explanation lay in the different relationship of sperm density to basal and peak FSH. Basal FSH remained fairly constant and only increased outwith the normal range when sperm density fell below 5 million/ml. A similar finding has been reported by Mauss & Börsch (1973) although the critical level of sperm density was 10 million/ml. The FSH response to LHRH (peak FSH) however showed a linear trend rising over the entire range of sperm density from 40 million to azoospermia. Thus patients with sperm density around 5–10 million/ml may have basal FSH within the normal range but exaggerated FSH response to LHRH—the group of patients that Lipschultz *et al.* (1977) selected for investigation. However, even in that study, basal FSH in the oligospermic patients was significantly elevated compared with controls, though the individual levels remained within the normal range. Furthermore, the magnitude of the LHRH response was proportional to basal FSH in all patient groups and controls. Their findings were therefore entirely compatible with those of the present study.

The elevated basal and peak LH found in patients with grade 3 and 4 histology confirmed the reports of de Kretser *et al.* (1972), Hunter *et al.* (1974) and Christiansen (1975). However, the testosterone levels in our patients were normal and a tendency for lower testosterone in patients with severe germ cell damage (de Kretser *et al.*, 1972) was not demonstrated. This apparent discrepancy is due to the exclusion of patients with chromosomal abnormalities (Klinefelter's and variants) and the smaller proportion of patients with more severe seminifereous tubular damage in the present study. The elevated LH with or without low testosterone may be the result of compensated Leydig cell failure, presumably caused by the same aetiological factors which affected the seminiferous tubular compartment. Recent evidence from *in vitro* studies (Rodriquez-Riqau *et al.*, 1978; Weiss *et al.*, 1978) confirmed abnormalities in Leydig cell functions in infertile men but the possibility that Leydig cell failure being a consequence of germ cell damage cannot be excluded.

Summarizing the clinical implications of the foregoing considerations: the most informative endocrine investigation in severly oligospermic (<5 million/ml) and azoospermic patients is a single estimation of basal FSH, raised levels of which are indicative of severe and probably irreversible germ cell damage. The response to LHRH stimulation confers very little additional information and is not indicated in routine management of male infertility. Measurement of LH and testosterone has limited value in patients without clinical evidence of androgen deficiency.

Having established the role of endocrine investigations it will be necessary to reconsider the indications for testicular biopsy in the assessment of male infertility. From the results of this study and that of Pryor *et al.* (1978), it is clear that in azoospermic patients, FSH can differentiate those with efferent duct obstruction from those with germ cell depletion

(Fig. 4). The latter group, with elevated FSH, will not require further investigation and can be advised to consider the alternatives of adoption or artificial insemination by donor at an early stage. For the azoospermic patients with normal FSH, scrotal exploration, vasogram and testicular biopsy should be performed to demonstrate the obstruction prior to epididymo-vasostomy. The importance of testicular biopsy, especially when the epididymides appear normal on exploration should be emphasized. This enable germ cell maturation arrest to be recognized as these patients are not suitable for epididymo-vasostomy. Baker *et al.* (1976) found that 52% of patients with germ cell arrest had elevated FSH levels. The classification of testicular histology in our study does not distinguish germ cell arrest as a separate category so that this finding could not be confirmed. For oligospermic patients with sperm density under 5 million/ml, elevated FSH has virtually the same implications as in azoospermic patients. If FSH is normal, testicular histology cannot be reliably predicted and biopsy is required if clinical decisions are made on the basis of histological changes in the semeniferous epithelium. For oligospermic patients with sperm density over 5 million/ml, neither testicular biopsy nor FSH estimation is required since the vast majority have normal levels of FSH and grade 1 or 2 histology (Fig. 4).

There were a number of patients that did not conform with predictions in this diagnostic scheme: five patients with elevated FSH but grade 1 and 2 histologies and five with normal FSH but grade 3 and 4 histologies. Valid explanations may be present for these apparent anomalies. Unilateral biopsies were performed in all except twelve cases. Where the two testes in a patient were of dissimilar size of consistency, the one considered more normal clinically was biopsied. Although it is generally held that testicular size is a good index of spermatogenesis in adults (Rifka & Sherins, 1978) several studies have emphasized that this may not be entirely reliable (Meinhard *et al.*, 1973; Pryor *et al.*, 1976 Guay *et al.*, 1977). In the present context, the five patients with normal FSH and grade 3 or 4 histologies may have more active spermatogenesis in the unbiopsied contralateral testis providing an adequate feedback to suppress FSH. The finding that all five patients had normal LH and testosterone and a normal or exaggerated response to LHRH makes the possibility of pituitary failure very unlikely.

One interesting finding in this study is that amongst the six patients with unilateral atrophic or absent testes, three had elevated basal FSH levels in spite of relatively good sperm production of over 15 million/ml. In a further two patients in this group the FSH response to LHRH was greatly exaggerated even though basal FSH was normal. This may be interpreted as a state of compensated spermatogenetic failure with the hypothalamic–pituitary unit being readjusted to a higher set point in response to the lower level of testicular feedback due to unilateral testicular atrophy. Whether compensatory hypertrophy occurs in the contralateral testis in these patients could not be assessed from the present study. Laron *et al.* (1975) postulated that compensatory hypertrophy occurs in the normal contralateral testis in patients with unilateral cryptorchidism, although the seminiferous tubular diameter remains unchanged. They also found FSH to be elevated in these patients with unilateral cryptorchidism. This was subsequently confirmed by Werder *et al.* (1976) and Lipschultz *et al.* (1976). In our patients however, unilateral testicular atrophy resulted not only from cryptochidism but also from variocoele, mumps orchitis, orchidectomy after a road traffic accident and radiation to the spine for ankylosing spondylitis. The clinical significance of our finding is that in the presence of obvious unilateral atrophic or absent testes from whatever

aetiology, elevated FSH levels are compatible with relatively good sperm production or even fertility and do not necessarily indicate severe germ cell failure.

Having considered these anomalous situations, there still remain a very small number of patients who did not fit into this diagnostic scheme. For these patients at least, one may have to postulate that the feedback signal for FSH (inhibin) is produced in some independent station within the seminiferous tubules whose functional integrity is dissociated from that of the germ cells (Leonard *et al.* 1972). This concept is compatible with the finding (Steinberger & Steinberger, 1976) that media from Sertoli cell culture can inhibit FSH secretion selectively in cultured pituitary cells.

REFERENCES

Aafjes, J.H., van der Vijver, J.C.M., Docter, R. & Schenk, P.E. (1977) Serum gonadotrophins, testosterone and spermatogenesis in subfertile men. *Acta Endocrinologica*, **86,** 651–658.

Alford, F.P., Baker, H.W.G., Burger, H.G., de Kretser, D.M., Hudson, B., Johns, M.W., Masterton, J.P., Patel, Y.C. & Rennie, G.C. (1973) Temporal patterns of integrated hormone levels during sleep and wakefulness II: FSH, LH, testosterone and oestradiol. *Journal of Clinical Endocrinology and Metabolism*, **37,** 848–854.

Baker, H.W.G., Bremner, W.J., Burger, H.G., de Kretser, D.M., Dulmanis, A., Eddie, L.W., Hudson, B., Keogh, E.J., Lee, V.W.K. & Rennie, G.C. (1976) Testicular control of follicle-stimulating hormone secretion. *Recent Progress in Hormone Research*, **32,** 429–469.

Chandley, A.C., Maclean, N., Edmond, P., Fletcher, J. & Watson, G.S. (1976) Cytogenetics and infertility in man. II. Testicular histology and meiosis. *Annals of Human Genetics (Lond.)*, **40,** 165–176.

Charny, C.W. (1940) Testicular biopsy and its value in male sterility. *Journal of the American Medical Association*, **115,** 1429–1432.

Christiansen, P. (1975) Studies on the relationship between spermatogenesis and urinary levels of follicle stimulating hormone and luteininzing hormone in oligospermic men. *Acta Endocrinologica*, **78,** 192–208.

Corker, C.S. & Davidson, D.W. (1978) Radioimmunoassay of testosterone on various biological fluids without chromatography. *Journal of Steroid Biochemistry*, **9,** 373–374.

de Kretser, D.M., Burger, H.G., Fortune, D., Hudson, B., Long, A.R., Paulsen, C.A. & Taft, H.P. (1972) Hormonal, histological and chromosomal studies in adult males with testicular disorders. *Journal of Clinical Endocrinology and Metabolism*, **35,** 392–401.

de Kretser, D.M., Burger, H.G. & Hudson, B. (1974) The relationship between germinal cells and serum FSH levels in males with infertility. *Journal of Clinical Endocrinology and Metabolism*, **38,** 787–793.

de Kretser, D.M., Burger, H.G. & Dumpys, R. (1975a) Serum LH and FSH response in 4 hour infusions of LHRH in normal men, Sertoli cell only, syndrome and Klinefelter's syndrome. *Journal of Clinical Endocrinology and Metabolism*, **41,** 876–886.

de Kretser, D.M., Burger, H.G., Hudson, R. & Keogh, E.J. (1975b) The hCG stimulation test in men with testicular disorders. *Clinical Endocrinology*, **4,** 591–596.

Eliasson, R. (1975) Analysis of semen. *Progress in Infertility* (eds S. J. Behrman & R. W. Kistner), 2nd edn. Little, Brown & Co., Boston.

Franchimont, P., Millet, D. Vendrely, E., Letawe, J. Legros, J.J. & Netter, A. (1972) Relationship between spermatogenesis and serum gonadotrophin levels in azoospermia and oligospermia. *Journal of clinical Endocrinology and Metabolism*, **34,** 1003–1008.

Franchimont, P., Demoulin, A. & Bourguignon, J.P. (1975) Clinical use of LHR test as a diagnostic tool. *Hormone Research*, **6,** 177–191.

Guay, A.T., Tuthill, R.J. & Woolf, P.D. (1977) Germinal cell aplasia: response of luteinizing hormone (LH), follicle stimulating hormone (FSH) and testosterone to LH/FSH releasing hormone with histopathologic correlation. *Fertility and Sterility*, **28,** 642–649.

Hunter, W.M., Edmond, P., Watson, G.S. & Maclean, N. (1974) Plasma LH and FSH levels in subfertile men. *Journal of Clinical Endocrinology and Metabolism*, **39,** 740–749.

Hunter, W.M. & Bennie, J.G. (1978) Minimization of non-specific serum responses in human pituitary gonadotrophin radioimmunoassays. *Journal of Endocrinology*, **80,** 59–68.

Isurgi, K., Wakabayaski, K. Fukutani, K., Takayasu, H., Tamaoki, B-I. & Okeda, M. (1973) Responses of serum luteininzing hormone and follicle-stimulating hormone levels to synthetic luteininzing hormone-

releasing hormone (LH-Rh) in various forms of testicular disorders. *Journal of Clinical Endocrinology and Metabolism*, **37**, 533–539.

JOHNSEN, S.G. (1970) Investigation into the feedback mechanism between spermatogenesis and gonadotrophin level in man. In *The Human Testic* (eds R. Rosemberg & C. A. Paulsen), pp. 231–248. Plenum Press, New York.

KJESSLER, B. & WIDE, L. (1973) Follicle stimulating hormone (FSH) and interstitial cell stimulating hormone (ICSH) in relation to gamete output in 643 males. *Acta Endocrinologica*, **72**, 243–256.

LARON, Z., DICKERMAN, Z., PRAGER-LEWIN, R., KERET, R. & KALABE, E. (1975) Plasma LH and FSH response to LRH in boys with compensatory testicular hypertrophy. *Journal of Clinical Endocrinology and Metabolism*, **40**, 977–981.

LEONARD, J.M., LEACH, R.B., COUTURE, M. & PAULSEN, C.A. (1972) Plasma and urinary follicle-stimulating hormone levels in oligospermia. *Journal of Clinical Endocrinology and Metabolism*, **34**, 209–214.

LIPSCHULTZ, L.I., CAMINOS-TORRES, R., GREENSPAN, C.S. & SNYDER, P.J. (1976) Testicular function after orchidopexy for unilateral undescended testis. *New England Journal of Medicine*, **295**, 15–18.

LIPSCHULTZ, L.I., GREENBERG, S.H., CAMINOS-TORRES, R. & SNYDER, P.J. (1977) Supranormal FSH response to gonadotrophin-releasing hormone in oligospermic men with a normal basal serum FSH concentration. *Clinical Endocrinology*, **7**, 103–109.

MARRIOT, F.H.C., (1974) *The interpretation of Multiple Observations*, pp. 32–43. Academic Press, New York.

MAUSS, J. & BÖRSCH, G. (1973) Immunochemical assay of follicle-stimulating and luteinizing hormone in the unconcentrated urine of sub or infertile males. *Acta Endocrinologia*, **74**, 631–641.

MECKLENBERG, R.S. & SHERINS, R.J. (1974) Gonadotrophin response to luteinizing hormone in men with germinal aplasia. *Journal of Clinical Endocrinology and Metabolism*, **38**, 1005–1008.

MEINHARD, E., MCRAE, C.U. & CHISOLM, G.D. (1973) Testicular biopsy in evaluation of male infertility. *British Medical Journal*, **iii**, 577–581.

NAFTOLIN, F., JUDD, H.L. & YEN, S.S.C. (1973) Pulsatile patterns of gonadotrophins and testosterone in man: the effects of clomiphene with and without testosterone. *Journal of Clinical Endocrinology and Metabolism*, **36**, 285–288.

NANKIN, H.R. & TROEN, P. (1971) Repetitive luteinizing hormonal elevations in serum of normal men. *Journal of Clinical Endocrinology and Metabolism*, **33**, 558–560.

PYROR, J.P. PUGH, R.C.B., CAMERON, K.M., NEWTON, J.R. & COLLINS, W.P. (1976) Plasma gonadotrophic hormones, testicular biopsy and seminal analysis in the men of infertile marriages. *British Journal of Urology*, **48**, 709–717.

PYROR, J.P., CAMERON, K.M., COLLINS, W.P., HIRSH, A.V., MAHONY, J.D.H., PUGH, R.C.B. & FITZPATRICK, J.M. (1978) Indications for testicular biopsy or exploration in azoospermia. *British Journal of Urology*, **50**, 591–594.

RIFKA, S.M. & SHERINS, R.J. (1978) Current concepts in evaluation of the infertile male. *Clinics in Obstetrics and Gynaecology*, **5**, 481–497.

RODRIGUEZ-RIGAU, L.H., WEISS, D.B., SMITH, K.D. & STEINBERGER, E. (1978) Suggestion of abnormal testicular steroidegeneses in some oligospermic men. *Acta Endocrinologica*, **87**, 400–412.

ROSEN, W.W. & WEINTRAUB, B.D. (1971) Monotropic increase of serum FSH correlates with low sperm count in young men with idiopathic oligospermia and aspermia. *Journal of Clinical Endocrinology and Metabolism*, **32**, 410–427.

ROULIER, R., MATTEI, A. & FRANCHIMONT, P. (1976) Intérêt et limites du dosage de FSH et LH avant et après stimulation par LH-RH dans les sterilities et hypogonadimes masculins. *Annales d'Endocrinologie (Paris)*, **37**, 407–427.

SANTEN, R.J. & BARDIN, C.W. (1973) Episodic luteininzing hormone secretion in man. *Journal of Clinical Investigation*, **52**, 2617–2628.

SMITH, K.D., TCHOLAKIAN, R.K., CHOWDHURY, M. & STEINBERGER, E. (1974) Rapid oscillations in plasma levels of testosterone, luteininzing hormone and follicle-stimulating hormone in men. *Fertility and Sterility*, **25**, 965–975.

STEINBERGER, A. & STEINBERGER, E. (1976) Secretion of an FSH-inhibition factor by cultured Sertoli cell. *Endocrinology*, **39**, 918–921.

WEISS, D.B., RODRIQUEZ-RIGAU, L.J., SMITH, K.D. & STEINBERGER, E. (1978) Leydig cell function in oligospermic men with varicocoele. *Journal of Urology*, **120**, 427–430.

WERDER, A., ILLIG, R., TORRESAMI, T., ZACHMANN, M., BAUMANN, P., OTT, F. PRADER, A. (1976) Gonadal function in young adults after surgical treatment of cryptorchidism. *British Medical Journal*, **ii**, 1357–1359.

Clinical Endocrinology (1982) **16**, 39–47

RAISED PLASMA OESTROGENS IN INFERTILE MEN WITH ELEVATED LEVELS OF FSH

F. C. W. WU, I. A. SWANSTON AND D. T. BAIRD

Department of Obstetrics and Gynaecology, University of Edinburgh, Centre for Reproductive Biology, 37 Chalmers Street, Edinburgh EH3 9EW

(Received 21 April 1981; revised 3 August 1981; accepted 11 August 1981)

SUMMARY

In twenty oligospermic or azoospermic patients with elevated plasma FSH, the mean concentrations of plasma oestrone sulphate (843 ± 233 pg/ml), oestrone ($54 \pm 10{\cdot}4$ pg/ml) and oestradiol ($46{\cdot}6 \pm 12{\cdot}6$ pg/ml) were found to be significantly higher than in twenty-one normal fertile men of comparable age (593 ± 220 pg/ml, $40{\cdot}6 \pm 8{\cdot}8$ pg/ml and $33{\cdot}1 \pm 10{\cdot}9$ pg/ml respectively). SHBG binding capacity was elevated in the infertile group (infertile $3{\cdot}35 \pm 0{\cdot}82 \times 10^{-8}$ M/l v. normal $2{\cdot}76 \pm 0{\cdot}89 \times 10^{-8}$ M/l) but the total plasma testosterone concentrations were comparable (infertile 5435 ± 1578 pg/ml v. normal 5046 ± 1102 pg/ml). Evidence was cited to support the view that Sertoli cells, in response to an unphysiological FSH stimulation, are a likely source of excessive oestrogen production. The possible significance of increased intra-testicular and circulating oestrogen in the altered state of testicular steroidogenic function in men with primary seminiferous tubular defects was discussed.

Although raised FSH has proved a useful diagnostic index of the state of the seminiferous epithelium (Hunter *et al.*, 1976; Wu *et al.*, 1981), in the majority of cases of male infertility, the underlying pathogenesis in the impairment of spermatogenesis remains unknown. With the realization that androgens are essential for spermatogenesis (Steinberger, 1971; Steinberger *et al.*, 1978) more attention has been focused on Leydig cell function in infertile males. Mean concentration of testosterone in infertile men has been found to be lower than normal (Rosen & Weinstraub, 1971; de Kretser *et al.*, 1972; Aloysio *et al.*, 1974; Purvis *et al.*, 1975; Nieschlag *et al.*, 1978) although this was not found by others (Ruder *et al.*, 1974; Lawrence & Swyer, 1974; Nankin *et al.*, 1977; Wu *et al.*, 1981). Some *in vitro* studies of testicular biopsy tissue from oligospermic men showed abnormalities in testicular steroidogenesis (Rodriguez-Rigau *et al.*, 1978; Oshima *et al.*, 1977) but, again, this was not confirmed (Nieschlag *et al.*, 1979). There was more general agreement in the finding of elevated LH levels which may be compatible with primary Leydig cell failure. Furthermore, the hCG response was subnormal in some severely oligospermic

Correspondence: Dr F. C. W. Wu, Royal Infirmary, Lauriston Place, Edinburgh EH3 9YN.

0300-0664/82/0100-0039$02.00

Details of the radioimmunoassay method for plasma oestrone and oestrone sulphate have already been described in full in a previous publication (Wu *et al.*, 1982). Plasma oestradiol, testosterone, LH and FSH concentrations were determined by radioimmunoassay according to the methods of Van Look *et al.* (1977), Corker & Davidson (1978) and Hunter & Bennie (1979) respectively. The sex-hormone binding capacity was measured by the method of Rosner modified by Anderson *et al.* (1976).

Statistical analyses were performed using Student's single-tailed *t* test.

RESULTS

The mean peripheral plasma concentrations of oestrone sulphate, oestrone and oestradiol in infertile males with elevated FSH were significantly higher than that in normal controls (Fig. 1). Plasma LH was elevated to a lesser extent than FSH, but testosterone remained indistinguishable from normal (Fig. 1).

In both azoospermic and oligospermic subgroups, the oestrogens and gonadotrophins remained significantly elevated from normal (Table 2). Comparison between azoospermic

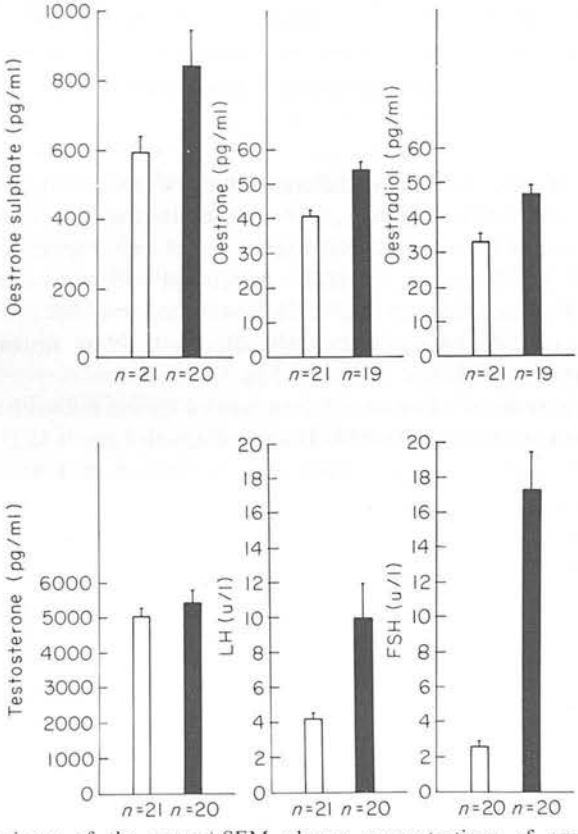


Fig. 1. Comparisons of the mean ± SEM plasma concentrations of oestrone sulphate ($P < 0{\cdot}0025$), oestrone ($P < 0{\cdot}0005$), oestradiol ($P < 0{\cdot}0025$), testosterone ($P > 0{\cdot}05$ NS), LH ($P < 0{\cdot}005$) and FSH ($P < 0{\cdot}0005$) between normal (□) and infertile (■) men.

plasma oestradiol but low oestrone concentrations in ten azoospermic men with elevated FSH levels. Nieschlag *et al.* (1978) however described a trend towards higher plasma oestradiol and lower testosterone concentrations with decreasing sperm density in infertile men.

In normal men, oestradiol and oestrone are predominantly derived from peripheral conversion of androgens (Baird *et al.*, 1969), while testicular secretion contributes a small but significant proportion of these oestrogens (Kelch *et al.*, 1972; Baird *et al.*, 1973; Weinstein *et al.*, 1974). Oestrone sulphate is almost entirely derived from peripheral conversion of oestrone and oestradiol (Longcope, 1972; Ruder *et al.*, 1972) with insignificant testicular secretion (Wu *et al.* 1982). In the present study, despite increased mean plasma levels of all three oestrogens in infertile men, the ratios between their concentrations were not different from normal. This implies that the dynamics of oestrogen production is qualitatively similar to normal men. Thus the excessive oestrogen production in infertile men could arise from either increased testicular secretion of oestradiol and oestrone and/or enhanced peripheral conversion of androgen precursors.

Low plasma testosterone (de Kretser *et al.*, 1972; Nieschlag *et al.*, 1978), normal androstenedione (Purvis *et al.*, 1975) and subnormal response to hCG stimulation (de Kretser *et al.*, 1975) are commonly found in infertile men. Sitterii & MacDonald (1973) suggested that increased testicular secretion accounted for the excessive oestrogen production in hypergonadotrophic patients since the rate of peripheral aromatization of androgens was normal. Recently, we found increased oestradiol concentrations in spermatic vein in patients with varicocoeles and elevated FSH (Wu *et al.*, 1982). Raised testicular oestradiol concentration (Damber & Bergh, 1980) and oestradiol production in response to hCG stimulation (de Kretser *et al.*, 1979) have been demonstrated in the experimentally-induced cryptorchid rat testis. Taken together, the evidence is compatible with increased testicular secretion of oestradiol (and oestrone) as a probable mechanism underlying the excessive oestrogen production in infertile men.

The observation that all three oestrogens were positively correlated with FSH suggests that elevation of this gonadotrophin may be important in enhancing testicular oestrogen secretion. This is supported by the finding that FSH-stimulated rat Sertoli cells in culture can aromatize testosterone and androstenedione to oestradiol (Dorrington & Armstrong, 1975; Rommerts *et al.*, 1978; 1979). However, the relative contribution of oestrogen synthesis by Leydig cells (Payne *et al.*, 1976; Valladares & Payne 1979a; 1979b; Canick *et al.*, 1979) compared to Sertoli cells remains to be defined.

A direct inhibitory action of oestrogens on Leydig cell function independent of the systemic suppression of LH has been demonstrated by a number of clinical and experimental studies (Baker *et al.*, 1973; Chen *et al.*, 1977; Jones *et al.*, 1978; Hseuh *et al.*, 1978; Van Buerden *et al.*, 1978). Thus Leydig cell function could be impaired subsequent upon a primary abnormality of spermatogenesis, increased pituitary FSH secretion and testicular over-production of oestrogens. This may explain the presence of Leydig cell dysfunction in infertile men (Rosen & Weintraub, 1971; de Kretser *et al.*, 1972; Aloysio *et al.*, 1974; Purvis *et al.*, 1975; Oshima *et al.*, 1977; Rodriquez-Rigau *et al.*, 1978; Nieschlag *et al.*, 1978) and in animals with experimentally-induced seminiferous tubular damage (Damber *et al.*, 1978; Rich *et al.*, 1979; Kerr *et al.*, 1979; de Kretser *et al.*, 1979; Main & Setchell, 1980). Furthermore, the consequent diminished supply of androgens to the Sertoli cells may cause a reduction in androgen binding protein production and a fall in intratubular androgens available for maintenance of spermatogenesis. This hypothetical series of

BAKER, H.W.G., BREMNER, W.J., BURGER, H.G., DE KRETSER, D.M., DULMANIS, A., EDDIE, L.W., HUDSON, B., KEOGH, E.J., LEE, V.W.K. & RENNIE, G.L. (1976) Testicular control of follicle-stimulating hormone secretion. *Recent Progress in Hormone Research*, **32,** 429–477.

CANICK, J.A., MAKRIS, A., GUNSALUS, G.L. & RYAN, K.J. (1979) Testicular aromatization in immature rats: localisation and stimulation after gonadotropin *in vivo*. *Endocrinology*, **104,** 285–288.

CHEN, Y.I., SHAW, M.J. & PAYNE, A.H. (1977) Steroid and FSH action on LH receptors and LH-sensitive testicular responsiveness during sexual maturation of the rat. *Molecular and Cellular Endocrinology*, **8,** 291–299.

CORKER, C.S. & DAVIDSON, D.W. (1978) Radioimmunoassay of testosterone in various biological fluids without chromatography. *Journal of Steroid Biochemistry*, **9,** 373–374.

DAMBER, J.E. & BERGH, A. (1980) Decreased testicular response to acute LH-stimulation and increased intratesticular concentration of oestradio-17β in the abdominal testes in cryptorchid rats. *Acta Endocrinologica*, **95,** 416–421.

DAMBER, J.E., BERGH, A. & JANSON, P.O. (1978) Testicular blood flow and testosterone concentration in the spermatic venous blood in rats with experimental cryptorchidism. *Acta Endocrinologica*, **88,** 611–618.

DORRINGTON, J.H. & ARMSTRONG, D.T. (1975) Follicle-stimulating hormone stimulates estradiol 17β synthesis in cultured Sertoli cells. *Proceedings of National Academy of Sciences of the USA*, **72,** 2677–2681.

DUFAU, M.L., HSEUH, A.J., CIGORRAGA, S., BAUKAL, A.J. & CATT, K.J. (1978) Inhibition of Leydig cell function through hormonal regulatory mechanisms. *International Journal of Andrology*, suppl. 2, 193–239.

FRANCHIMONT, P., VERSTRAELENPROYARD, M.T., HAZEEHAGOLSTEIN, C.R.A., DEMOULIN, A., BOURGUINON, J.P. & HUSTIN, J. (1979) Inhibin—from concept to reality. *Vitamins and Hormones*, **37,** 243–302.

HSEUH, A.J.W., DUFAU, M.L. & CATT, K.J. (1978) Direct inhibitory effects of estrone on Leydig cell function of hypophysectomized rats. *Endocrinology*, **103,** 1096–1102.

HUNTER, W.M. & BENNIE, J.G. (1979) Reduction of non-specific serum responses in human pituitary gonadotrophin radioimmunoassay. *Journal of Endocrinology*, **80,** 59–68.

HUNTER, W.M., EDMOND, P., WATSON, G.S. & MACLEAN, N. (1974) Plasma LH and FSH levels in subfertile men. *Journal of Clinical Endocrinology and Metabolism*, **39,** 740–749.

JONES, T.H., FANG, V.S., LANDAU, R.L. & ROSENFIELD, R. (1978) Direct inhibition of Leydig cell function by oestradiol. *Journal of Clinical Endocrinology and Metabolism*, **47,** 1368–1373.

KELCH, R.P., JENNER, M.R., WEINSTEIN, R. KAPLAN, S. & GRUMBACH, M.M. (1972) Oestradiol and testosterone secretion by human, simian and canine testes in males with hypogonadism and in male pseudohermaphrodites with the feminizing testes syndrome. *Journal of Clinical Investigation*, **51,** 824–830.

KERR, J.B., RICH, K.A. & DE KRETSER, D.M. (1979) Alterations of the fine structure and androgen secretion of the interstitial cells in the experimentally cryptorchid rat testis. *Biology of Reproduction*, **20,** 409–422.

DE KRETSER, D.M. (1979) Endocrinology of male infertility. *British Medical Bulletin*, **35,** 187–192.

DE KRETSER, D.M., BURGER, H.G., FORTUNE, D., HUDSON, B., LONG, A.R., PAULSEN, C.A. & TAFT, H.P. (1972) Hormonal histological and chromosomal studies in adult males with testicular disorders. *Journal of Clinical Endocrinology and Metabolism*, **35,** 392–401.

DE KRETSER, D.M., BURGER, H.G., HUDSON, B. & KEOGH, E.J. (1975) The hCG stimulation test in men with testicular disorders. *Clinical Endocrinology*, **4,** 591–596.

DE KRETSER, D.M., SHARPE, R.M. & SWANSTON, I.A. (1979) Alterations in steroidogenesis and human chorionic gonadotropin binding in the crytorchid rat testis. *Endocrinology*, **105,** 135–138.

KULIN, H.E., & REITER, E.O. (1972) Gonadotropin suppression by low dose oestrogen in men evidence for differential effects upon FSH and LH. *Journal of Clinical Endocrinology and Metabolism*, **35,** 836–839.

LACROIX, A., MCKENNA, T.J. & RABINOWITZ, D. (1978) Sex steroid modulation of gonadotrophin in normal men and in androgen insensitivity syndrome. *Journal of Clinical Endocrinology, and Metabolism*, **48,** 235–240.

LAWRENCE, D.M. & SWYER, G.I. (1974) Plasma testosterone and testosterone binding affinities in men with impotence, oligospermia, azoospermia and hypogonadism. *British Medical Journal*, **i,** 349–351.

LONGCOPE, C. (1972) The metabolism of estrone sulphate in normal males. *Journal of Clinical Endocrinology and Metabolism*, **34,** 113–122.

MAIN, S.J., DAVIES, R.V. & SETCHELL, B.P. (1980) Effects of passive immunization against testosterone in serum and pituitary gonadotrophins: implications for the relative importance of androgens and inhibition in the control of gonadotrophin secretion in the male rat. *Journal of Endocrinology*, **86,** 135–146.

MAIN, S.J. & SETCHELL, B.P. (1980) Responsiveness of the pituitary gland to androgens and of the testis to gonadotrophins following damage to spermatogenesis in rats. *Journal of Endocrinology*, **87,** 445–454.

STEINBERGER, E. (1971). Hormonal control of mammalian spermatogenesis. *Physiological Review*, **51,** 1–22.

STEINBERGER, E., STEINBERGER, A. & SANBORN, B.M. (1978) Molecular mechanisms concerned with hormonal control of the seminiferous epithelium. In *Recent Progress in Andrology*. Proceedings of Serono Symposium. Vol. 14, (eds A. Fabbrini & E. Steinberger), pp. 143–178. Academic Press, London.

VALLADARES, L.E. & PAYNE, A.H. (1979a) Acute stimulation of aromatization in Leydig cells by human chronic gonadotrophin *in vitro*. *Proceedings of the National Academy of Science of the USA*, **76,** 4460–4463.

VALLADARES, I.E. & PAYNE, A.H. (1979b) Induction of testicular aromatization of luteinizing hormone in mature rats. *Endocrinology*, **105,** 431–436.

VAN BUERDEN, W.M.D., ROODNAT, B. & VAN DER MOLEN, H.J. (1978) Effects of oestrogen and FSH on LH stimulation of steroid production by testis Leydig cells from immature rats. *International Journal of Andrology*, suppl. *2*, 374–383.

VAN LOOK, P.F.A., HUNTER, W.M., CORKER, C.S. & BAIRD, D.T. (1977) Failure of positive feedback in intact men and in male pseudohermaphrodites with the syndrome of testicular feminization. *Clinical Endocrinology*, **7,** 353–366.

VERMEULEN, A., VERDONCK, L., VAN DER STRAETEN, M. & ORIE, N. (1969) Capacity of the testosterone-binding globulin in human plasma and influence of specific binding of testosterone on the metabolic clearance rate. *Journal of Clinical Endocrinology and Metabolism*, **29,** 1470–1480.

WEINSTEIN, R.L., KELCH, R.P., JENNER, M.R., KAPLAN, S.L. & GRUMBACH, M.M. (1974) Secretion of unconjugated androgens and estrogens by the normal and abnormal human testis before and after human chorionic gonadotropin. *Journal of Clinical Investigation*, **53,** 1–6.

WIELAND, R.G., ZORN, E.M., MILES, K. & HALLBERG, M.C. (1974) Endocrinologic evaluation of oligospermic males: determination of dihydrotestosterone and estradiol. *Clinical Research*, **22,** 634A.

WU, F.C.W., EDMOND, P., RAAB, G. & HUNTER, W.M. (1981) Endocrine assessment of the subfertile male. *Clinical Endocrinology*, **14,** 493–507.

WU, F.C.W., SWANSTON, I.A., HARGREAVE, T.B. & BAIRD, D.T. (1982) Evidence that the human testis does not secrete oestrone sulphate. *Journal of Endocrinology*, in press.

J. Endocr. (1982), **92**, 185–194 *Printed in Great Britain*
0022–0795/82/0092–0185 $02.00/0

HUMAN TESTIS DOES NOT SECRETE OESTRONE SULPHATE

F. C. W. WU*, I. A. SWANSTON, T. B. HARGREAVE† AND D. T. BAIRD

Department of Obstetrics and Gynaecology and †Department of Surgery/Urology, University of Edinburgh, Edinburgh, EH3 9YW

(*Received* 11 *March* 1981)

SUMMARY

The concentrations of five steroids in samples of spermatic venous blood collected from 17 men undergoing ligation of varicocoeles were compared with those in samples from the antecubital vein. There was evidence of testicular secretion of testosterone, androstenedione, oestradiol-17β and oestrone, since the ratios of the mean concentrations in spermatic venous plasma to those in peripheral venous plasma were 77·2, 9·1, 28·7 and 1·6 respectively. The testicular secretion of oestrone sulphate was minimal; the ratio of the mean concentrations in spermatic and peripheral plasma was 1·07. These results support the view derived from isotope dilution studies that almost all oestrone and oestrone sulphate in the circulation is derived from peripheral conversion of other precursor steroids.

INTRODUCTION

Direct testicular secretion of oestrone and oestradiol in man has been inferred from the fact that the concentrations of these oestrogens in plasma from the spermatic vein are higher than in the peripheral vein (Kelch, Jenner, Weinstein, Kaplan & Grumbach, 1972; Baird, Galbraith, Fraser & Newsam, 1973). It has been suggested that oestrone sulphate, the most abundant oestrogen in peripheral plasma (Brown & Smyth, 1971), may also be secreted from the human testis (Baird, Horton, Longcope & Tait 1968; Hembree, Bardin & Lipsett, 1969) as in the stallion (Raeside, 1969). However, isotope dilution studies have indicated that the entire blood production of oestrone sulphate can be accounted for by peripheral conversion of unconjugated oestrone and oestradiol (Purdy, Engel & Oncley, 1961; Longcope, 1972; Ruder, Loriaux & Lipsett, 1972). Because of significant diurnal variation in the secretion of androgens, the contribution to oestrogens by the peripheral conversion of androstenedione and testosterone measured under steady state conditions may have been overestimated (Baird, Horton, Longcope & Tait, 1969).

In this study we have attempted to determine directly the testicular secretion of oestrogens by measurement of the concentrations of oestrone, oestradiol-17β and oestrone sulphate in spermatic venous blood of men undergoing surgery for operative repair of varicocoeles.

PATIENTS, MATERIALS AND METHODS

Patients

Seventeen patients (age 18–34 years, mean 26·2 ± 4·4 (S.D.) years) in good general health who had received no regular medications before surgery underwent ligation of the left spermatic vein for treatment of varicocoeles. During the operation, which was performed

* Present address: Royal Infirmary, Edinburgh, EH3 9YW.

$NaHCO_3$ and with 1 ml sterile distilled water and evaporated to dryness. The residue was redissolved in 500 µl phosphate–gelatin buffer and kept at −20 °C until assay. Two 100-µl portions from each sample were assayed and 200 µl counted for estimation of recovery.

Further purification of solvolysed extract by celite column chromatography

Oestrone sulphate in plasma from postmenopausal females and normal males was processed as described above and the oestrone obtained was assayed with and without purification with celite column chromatography (Thorneycroft, Ribiero, Stone & Tillson, 1973). Celite 545 (Johns–Manville Products Corporation, Richmond) was heated to 600 °C for 18 h to remove contaminants and was cooled in a desiccator. It was mixed thoroughly with ethylene glycol (2:1, w/v) and the columns were packed dry in 5-ml graduated disposable pipettes to a height of 6 cm. After washing the columns twice with 5 ml iso-octane, the residues were applied in 0·5 ml iso-octane and then again washed with 2 ml and then 3·5 ml iso-octane. The oestrone fraction was eluted with 4 ml 15% ethyl acetate in iso-octane which was evaporated under N_2. The residues were redissolved in 200 µl buffer of which 100 µl was assayed and 50 µl counted for recovery.

Radioimmunoassay

The antiserum was raised in ewes against oestrone-6-(O-carboxymethyl)oxime–bovine serum albumin (Martensz, Scaramuzzi & Van Look, 1979). Oestrone extracted before solvolysis and unconjugated oestrone liberated by solvolysis of the same samples were analysed together in one assay. To each sample antiserum (100 µl, 1:10 000 dilution in buffer) was added and the contents of the tubes were mixed and equilibrated at room temperature for 1 h. [^{3}H]Oestrone (5000 counts/min in 0·1 ml buffer) was added, mixed and the samples left overnight at 4 °C. Unbound oestrogen was removed by adding 1 ml dextran-coated charcoal suspension; the mixture was kept at 0 °C for 15 min in an ice bath. The charcoal was separated by centrifugation at 1000 *g* for 10 min at 4 °C and the supernatant fraction decanted into counting vials.

Standard curves were obtained from samples containing known quantities of oestrone (0–1·85 pmol) processed by the same procedure as the unknown samples. The mass of oestrone in the latter was extrapolated directly from the standard curves and corrected for procedural losses.

Liquid scintillation counting

All samples were counted in 10 ml scintillation fluid containing 2,5-diphenyloxazole (45 mmol) and 1,4-di-2,5-phenylozazolyl-benzene (2 mmol) in 2·5 litres sulphur-free toluene and 1·25 litres Triton X-100 (all from Koch–Light Laboratories Ltd, Colnbrook). Tritium was measured in a Packard Tri-Carb Liquid Scintillation spectrometer (model 3320) with a counting efficiency for tritium of 54%. Sufficient counts were recorded to give a counting error of less than 5%.

Radioimmunoassay of other hormones

Oestradiol-17β was measured by the method of Van Look, Hunter, Corker & Baird (1977). The intra- and interassay variations were 9·3 and 10% respectively and the limit of detection was 37 pmol/l.

Androstenedione was measured by the method of Baird, Burger, Heavon-Jones & Scaramuzzi (1974) after purification by alumina column chromatography (McNatty, Baird, Bolton, Chambers, Corker & McLean, 1976). The intra- and interassay variations were 5·7 and 5·2% respectively and the limit of detection was 0·52 nmol/l.

Testosterone was measured by the method of Corker & Davidson (1978) without chromatography. The intra- and interassay variations were 7·6 and 11·5% respectively and the limit of detection was 0·56 nmol/l.

Table 1. *Concentrations of oestrone sulphate, oestrone, oestradiol, androstenedione and testosterone in spermatic (S) and antecubital (P) venous plasma and gonadotrophins in antecubital venous plasma of men with varicocoeles*

Subjects	Age (years)	Sperm density (10^6/ml)	Oestrone sulphate (nmol/l)		Oestrone (pmol/l)		Oestradiol (pmol/l)		Androstenedione (nmol/l)		Testosterone (nmol/l)		Gonadotrophins (units/l)	
			S	P	S	P	S	P	S	P	S	P	LH	FSH
1 G.P.	25		2·0	2·6	115	130	426	173	6·7	4·1	177	15	9·9	7·5
2 E.M.	22		2·8	2·6	156	193	426	180	3·7	3·9	209	10	3·2	1·9
3 D.D.	18		2·8	2·1	756	193	—	195	135·8	3·6	6644	15	5·6	2·6
4 A.C.	27		2·7	2·7	467	144	16603	132	135·2	4·5	3000	14	10·8	16·3
5 B.B.	33		1·3	1·1	126	174	283	143	17·1	2·1	56	27	4·2	1·6
6 D.A.	27	1·0	2·1	2·0	189	178	11386	129	8·2	3·6	100	11	8·4	9·2
7 D.M.	23	167·5	2·8	2·8	441	163	331	107	19·3	5·8	461	10	2·2	1·0
8 P.D.	28	1·5	2·4	2·1	137	148	331	195	10·8	5·0	13	10	11·6	3·3
9 A.D.	24	26·0	5·0	5·1	307	252	283	261	6·0	2·8	60	15	10·1	5·9
10 W.P.	33		2·0	1·8	233	189	7353	154	49·2	5·8	2251	14	14·8	7·1
11 A.D.	19		3·7	3·0	319	244	1518	99	25·3	4·8	643	12	—	—
12 R.B.	30	122·0	2·5	2·2	415	252	7353	129	83·3	4·0	2679	28	7·9	2·4
13 B.W.	27	7·3	1·9	1·6	363	215	21345	143	130·3	4·0	182	12	8·7	6·3
14 R.F.	23	20·2	3·1	3·2	367	189	2371	206	41·1	5·9	707	17	3·5	5·2
15 H.S.	34	23·3	3·4	2·6	137	185	426	132	5·5	13·9	11	21	4·7	3·5
16 J.D.	26		1·6	1·5	159	107	1566	184	90·2	5·5	3000	15	9·5	6·2
17 J.C.	27		3·1	3·3	278	111	—	268	—	10·2	686	23	9·0	5·3
Mean	26·2		2·65	2·48	292	180	4798	167	48·0	5·27	1228	15·9	7·76	5·2
(S.D.)	(4·4)		(0·85)	(0·88)	(163)	(43)	(6504)	(47)	(4·85)	(2·76)	(1723)	(5·56)	(3·40)	(3·78)
P value (paired *t*-test)			<0·05		<0·01		<0·025		<0·005		<0·001		—	
Ratio of mean concentrations in spermatic venous plasma and peripheral venous plasma			1·07		1·6		28·7		9·1		77·2		—	

the assay was achieved by the solvolysis procedure and the low cross-reaction of the antiserum with possible interfering steroids. This is further substantiated by the similarity of results obtained with or without celite column chromatography. The accuracy and precision were within the acceptable limits for radioimmunoassays. The sensitivity was adequate for the range of values studied in the present investigation and values for water and steroid-free plasma were below the limits of detection of the method. The mean peripheral venous concentration of oestrone sulphate in 17 men undergoing varicocoele repair was $2{\cdot}48 \pm 0{\cdot}88$ (S.D.) nmol/l. This is comparable to values given in previous reports, for example, Hawkins & Oakey (1974): $1{\cdot}95 \pm 0{\cdot}80$ nmol/l; Wright, Collins, Musey & Preedy (1978): $2{\cdot}50 \pm 0{\cdot}17$ nmol/l; Franz, Watson & Longcope (1979): $1{\cdot}24 \pm 0{\cdot}07$ nmol/l; and Towobola, Crilly & Oakey (1980): $3{\cdot}20 \pm 0{\cdot}61$ nmol/l.

The demonstration of adrenal secretion of dehydroepiandrosterone sulphate (Baulieu, Corpechot, Dray, Emiliozzi, Lebeau, Mauvais-Jarvis & Robel, 1965) and the finding that sulphated steroids can be metabolized without prior hydrolysis of the sulphate moeity (Lieberman, 1967) stimulated interest in investigating whether phenolic steroid conjugates are also secreted by steroid-producing glands or formed solely as a result of peripheral conversion of precursors. The present study demonstrated an insignificant testicular vein–peripheral vein concentration gradient for oestrone sulphate indicating, if concentration in the spermatic artery is equated with that in the peripheral vessels, that there is no testicular secretion of this conjugated oestrogen despite its relative abundance in the circulation. Using the transfer constants (defined as the fraction of precursor which enters the blood *de novo* as the relevant product) from Ruder *et al.* (1972) and Longcope (1972) it was possible to estimate, from the plasma concentrations in the present studies, that nearly all of the plasma oestrone sulphate is derived from oestrone and oestradiol. Since the error in such calculations is high, it is possible that the adrenal gland is a further source of oestrone sulphate. Sneddon & Marrian (1963) showed that bovine adrenal tissue can synthesize oestrone sulphate *in vitro*, but whether there is significant synthesis *in vivo* and secretion of oestrone sulphate by human adrenals remain to be determined. Thus on present evidence, unlike dehydroepiandrosterone sulphate, oestrone sulphate is not an important precursor for unconjugated oestrogens under physiological conditions in man.

Testicular secretion of testosterone, androstenedione, oestradiol and oestrone was confirmed by the spermatic–peripheral vein concentration differentials found in the present study. The spermatic vein concentrations of testosterone, androstenedione and oestradiol are comparable to those given in previous reports (Laatikainen, Laitinen & Vihko, 1971; Weinstein, Kelch, Jenner, Kaplan & Grumbach, 1974; Fiorelli, Borrelli, Forti, Gonnelli, Pazzagli & Serio, 1976; Hammond, Ruokonen, Kontturi, Kosbela & Vihko, 1977; Pirke, Sintermann & Doerr, 1977; de la Torre, Noren, Hedman & Diczfalusy, 1978). The spermatic vein concentration of oestrone, however, is considerably lower than that reported by Baird *et al.* (1973) and Weinstein *et al.* (1974) but more comparable to that found by Longcope, Widrich & Swain (1972). In this latter study, unexpectedly low spermatic vein concentrations of androstenedione, testosterone and oestradiol were also found, suggesting that the retrograde approach via the inferior vena cava and renal vein might have introduced a degree of dilution in the spermatic vein effluent. This is unlikely to be the case in the present study since the approach to the spermatic vein was direct and the spermatic vein concentrations of other steroids are comparable to those given in other published reports. The correlation of spermatic-peripheral vein concentration ratios of the four unconjugated steroids further substantiates the validity of the present results. The considerable variations in the concentrations of the steroids measured in spermatic venous plasma (coefficient of variation 33–134%) are not unexpected in view of the episodic nature of testicular hormone secretion (Smith, Tcholakian, Chowdhury & Steinberger, 1974) and the variability inherent in the anatomical level of operative approach to the spermatic vein, the duration of, and the negative pressure applied in, blood sampling. Where the rate of

Comhaire, F. & Vermeulen, A. (1975). Plasma testosterone in patients with varicocoele and sexual inadequacy. *Journal of Clinical Endocrinology and Metabolism* **40**, 824–829.

Corker, C. S. & Davidson, D. W. (1978). Radioimmunoassay of testosterone on various biological fluids without chromatography. *Journal of Steroid Biochemistry* **9**, 373–374.

Eik-Nes, K. B. (1970). Synthesis and secretion of androstenedione and testosterone. In *The androgens of the testis*, pp. 10–14. Ed. K. B. Eik-Nes. New York: Marcel Dekker Inc.

Fiorelli, G., Borrelli, D., Forti, G., Gonnelli, P., Pazzagli, M. & Serio, M. (1976). Simultaneous determination of androstenedione, testosterone and dihydrotestosterone in human spermatic and peripheral venous plasma. *Journal of Steroid Biochemistry* **7**, 113–116.

Franz, C., Watson, D. & Longcope, C. (1979). Oestrone sulphate and dehydroepiandrosterone sulphate concentrations in normal subjects and men with cirrhosis. *Steroids* **34**, 563–573.

Hammond, G. L., Ruokonen, A., Kontturi, M., Kosbela, E. & Vihko, R. (1977). The simultaneous radioimmunoassay of seven steroids in human spermatic and peripheral venous blood. *Journal of Clinical Endocrinology and Metabolism* **45**, 16–24.

Hawkins, R. A. & Oakey, R. E. (1974). Estimation of oestrone sulphate, oestradiol-17β and oestrone in peripheral plasma: concentrations during menstrual cycle and in men. *Journal of Endocrinology* **60**, 3–17.

Hembree, W. C., Bardin, C. W. & Lipsett, M. B. (1969). A study of estrogen metabolic clearance rates and transfer factors. *Journal of Clinical Investigation* **48**, 1809–1819.

Hunter, W. M. & Bennie, J. G. (1979). Minimization of non-specific serum responses in human pituitary gonadotrophin radioimmunoassays. *Journal of Endocrinology* **80**, 59–68.

Kelch, R. P., Jenner, M. R., Weinstein, R., Kaplan, B. L. & Grumbach, M. M. (1972). Estradiol and testosterone secretion by human simian and canine testes in males with hypogonadism and in male pseudo-hermaphrodites with the feminizing testes syndrome. *Journal of Clinical Investigation* **51**, 824–830.

Laatikainen, T., Laitinen, E. A. & Vihko, R. (1971). Secretion of free and sulfate-conjugated neutral steroids by the human testis. Effects of administration of human chorionic gonadotrophin. *Journal of Clinical Endocrinology and Metabolism* **32**, 59–64.

Lieberman, S. (1967). Steroid sulphates as biosynthetic intermediates. *Excerpta Medica International Congress Series* **132**, 22–36.

Longcope, C. (1972). The metabolism of estrone sulfate in normal males. *Journal of Clinical Endocrinology and Metabolism* **34**, 113–122.

Longcope, C., Widrich, W. & Swain, C. T. (1972). The secretion of oestrone and oestradiol-17β by human testis. *Steroids* **20**, 439–448.

McNatty, K. P., Baird, D. T., Bolton, A., Chambers, P., Corker, C. S. & McLean, H. (1976). Concentration of oestrogens and androgens in human ovarian venous plasma and follicular fluid throughout the menstrual cycle. *Journal of Endocrinology* **71**, 77–85.

Martensz, N. D., Scaramuzzi, R. J. & Van Look, P. F. A. (1979). Plasma concentrations of luteinizing hormone and follicle-stimulating hormone during anoestrus in ewes actively immunized against oestradiol-17β, oestrone or testosterone. *Journal of Endocrinology* **81**, 261–269.

Pirke, K. M., Sintermann, R. & Doerr, P. (1977). Testosterone and testosterone precursors in the spermatic vein of young and old men. *Acta Endocrinologica* **84**, Suppl., 78–79.

Purdy, R. H., Engel, L. L. & Oncley, J. L. (1961). The characterization of oestrone sulphate from human plasma. *Journal of Biological Chemistry* **236**, 1043–1050.

Raboch, J. & Starka, L. (1971). Hormonal testicular activity in men with a varicocoele. *Fertility and Sterility* **22**, 152–155.

Raeside, J. I. (1969). The isolation of estrone sulfate and estradiol sulfate from stallion testis. *Canadian Journal of Biochemistry* **47**, 811–815.

Rege, N., Phadke, A., Bhatt, J., Khatri, N., Sheth, A., Joski, W. & Vaidya, R. (1979). Serum gonadotrophins and testosterone in infertile patients with varicocoele. *Fertility and Sterility* **31**, 413–416.

Ruder, H. J., Loriaux, L. & Lipsett, M. B. (1972). Estrone sulfate: production rate and metabolism in men. *Journal of Clinical Investigation* **51**, 1020–1033.

Schiff, I., Wilson, E., Newton, R., Shane, J., Kates, R., Ryan, K. J. & Naftolin, F. (1976). Serum luteinizing hormone, follicle stimulating hormone and testosterone responses to gonadotrophin-releasing factor in males with varicocoeles. *Fertility and Sterility* **27**, 1059–1061.

Smith, K. D., Tcholakian, R. K., Chowdhury, M. & Steinberger, E. (1974). Rapid oscillations in plasma levels of testosterone, luteinizing hormone and follicle stimulating hormone in men. *Fertility and Sterility* **25**, 965–975.

Sneddon, A. & Marrian, G. F. (1963). Sulphation of oestrone *in vitro* by bovine adrenal tissue. *Biochemical Journal* **86**, 385–388.

Swerdloff, R. S. & Walsh, P. C. (1975). Pituitary and gonadal hormones in patients with varicocoele. *Fertility and Sterility* **26**, 1006–1012.

Thorneycroft, I. H., Ribiero, W. O., Stone, S. C. & Tillson, S. A. (1973). A radioimmunoassay of androstenedione. *Steroids* **21**, 111–122.

Towobola, O. A., Crilly, R. C. & Oakey, R. E. (1980). Oestrone sulphate in plasma from postmenopausal women and the effects of oestrogen and androgen therapy. *Clinical Endocrinology* **13**, 461–471.

men (de Kretser *et al.*, 1975). The weight of available evidence would support the presence of Leydig cell dysfunction in men with primary defects of spermatogenesis. The mechanism underlying this observation is at present unknown.

In man, the normal testis secretes oestrogens (Kelch *et al.*, 1972; Baird *et al.*, 1973; Weinstein *et al.*, 1974), although their physiological function is unclear. Dufau *et al.* (1978) suggested that oestrogens may have an intra-testicular role in the local control of Leydig cell function. Dorrington & Armstrong (1975) demonstrated the ability of FSH-stimulated rat Sertoli cells to form oestradiol from testosterone and androstenedione *in vitro*. Oestrogens may therefore be important in the pathogenesis of Leydig cell dysfunction in infertile men.

We report here in an investigation of the levels of circulating oestrogens in infertile men with pathologically-elevated FSH. Oestrone sulphate, oestrone and oestradiol were measured in plasma to provide comprehensive indices of oestrogenic function in men with primary disorders of seminiferous tubules.

PATIENTS AND METHODS

Peripheral venous blood samples were obtained from twenty patients attending the Male Subfertility Clinic, Royal Infirmary, Edinburgh. These patients were selected by the finding of one or more elevated plasma FSH level (normal male range 1·6–6·7 u/l) in association with repeated azoospermia or oligospermia (sperm density under 10 million/ml). Thirteen patients were oligospermic with mean sperm density of $3{\cdot}1 \pm 2{\cdot}3$, range 0·25–8·5 million/ml. Seven patients were azoospermic. The mean age of this infertile group ($n = 20$) was $28{\cdot}9 \pm 4{\cdot}3$ years. All patients had a normal 46 XY karyotype and normally developed secondary sexual characteristics. Details of the clinical findings are summarized in Table 1.

The control group was represented by thirteen men whose wives had delivered normal infants within the previous $4{\cdot}6 \pm 4$ months in the Simpson's Memorial Maternity Pavilion, Edinburgh. No semen analysis was performed in this group. Blood samples were also obtained from eight male partners of infertile couples attending the Gynaecological Infertility Clinic, Royal Infirmary, Edinburgh; they were considered fertile by virtue of repeatedly normal semen analyses (mean sperm density $= 181{\cdot}5 \pm 84{\cdot}2$, range 86–327 million/ml) and the absence of any abnormalities in the history or examination. The mean age of the normal fertile control groups ($n = 21$) was $31{\cdot}8 \pm 4{\cdot}1$ years.

Table 1. Clinical findings in twenty infertile men*

History		Examination	
Mumps orchitis	1	L. varicocoele	2
R. inguinal herniorrhaphy	1	R. undescended testis	1
Bilateral orchidopexy	3	Small atrophic testes	
Unilateral testicular torsion	1	Unilateral	1
		Bilateral	7

* Some patients had more than one of the above abnormalities. One azoospermic and five oligospermic patients did not have any abnormalities in both history or examination.

Table 2. Comparisons of mean plasma levels of oestrone sulphate, oestrone, oestradiol, SHBG, LH and FSH in azoospermic, oligospermic patients and normal controls

Study Groups		Oestrone Sulphate (pg/ml)	Oestrone (pg/ml)	Oestradiol (pg/ml)	Testosterone (pg/ml)	SHBG ($\times 10^{-8}$ m/l)	LH (u/l)	FSH (u/l)
Azoospermic	Mean	829	52·6	50·3	5328	2·96	14·4	27·2
(A)	SD	225	8·6	12·1	1298	0·63	1·4	10·4
	Range	539–1225	36–64	40–76	3974–8024	2·37–4·0	4·4–46·4	12·7–42.7
	N	7	7	6	7	4	7	7
Oligospermic	Mean	851	54·8	44·8	5492	3·60	7·4	12·0
(O)	SD	237	11·2	12·5	1707	0·88	1·5	33
	Range	422–1423	31–77	21–67	2765–8025	2·7–5·0	5·3–11·1	7·2–20·7
	N	13	13	13	13	8	13	13
Normal	Mean	593	40·6	33·1	5046	2·76	4·2	2·6
(N)	SD	220	8·8	10·9	1102	0·89	1·6	1·4
	Range	369–1230	22–58	17–59	3457–7160	1·20—4·74	1·9–8·3	1·0–5·5
	N	21	20	21	21	16	21	20
Statistical	A v O	NS	NS	NS	NS	NS	$<0·05$	$<0·0005$
difference	A v N	$<0·025$	$<0·005$	$<0·005$	NS	NS	$<0·0025$	$<0·0005$
P	O v N	$<0·0025$	$<0·0005$	$<0·005$	NS	$<0·05$	$<0·0005$	$<0·0005$

NS = not significant when $P \geqslant 0·05$.

and oligospermic patients showed no difference with respect to the three oestrogens and testosterone. LH and FSH, however, were higher in the azoospermic group. Mean SHBG-binding capacity was significantly higher in eight oligospermic patients than controls but the small number of azoospermic patients did not show any difference from either normal or oligospermic men (Table 2). Combined together as one infertile group, SHBG binding capacity was significantly elevated from normal ($3·35 \pm 0·82$ v. $2·76 \pm 0·89 \times 10^8$ M, $P < 0·05$, not shown in Fig. 1).

A significant but weak positive correlation was obtained between FSH and oestrone ($r = 0·3946$), oestrone sulphate ($r = 0·3457$) and oestradiol ($r = 0·3221$) when the patient and control groups were combined. Correlations between LH and oestrone sulphate ($r = 0·3686$), LH and FSH ($r = 0·6278$) were also present.

Although the mean absolute levels of all three oestrogens were higher in the infertile group, the ratios of oestrone sulphate to oestrone (15·6 : 1) oestrone sulphate to oestradiol (18·1 : 1) and oestrone to oestradiol (1·2 : 1) have remained similar to that in the normal controls—14·6 : 1, 17·9 : 1 and 1·2 : 1 respectively.

DISCUSSION

This is the first report of increased concentrations of plasma oestrone sulphate, oestrone and oestradiol in infertile men with pathologically raised levels of FSH. It contrasts with the studies of Wieland *et al.* (1974) and Nankin *et al.* (1977) who found normal concentration of oestradiol in a similar group of patients. Purvis *et al.* (1975) demonstrated normal

events may form a vicious circle which aggravates or perpetuates the initial insult to the seminiferous epithelium (de Kretser, 1979).

It is noteworthy that concurrent with the rise in plasma oestrogens in infertile men with elevated FSH, SHBG-binding capacity was also significantly increased. As excessive endogenous on exogenous oestrogen is well-known to increase SHBG concentration (Pearlman *et al.*, 1967; Migeon *et al.*, 1968; Vermeulen *et al.*, 1969), this observation provides further supportive evidence for the principle findings in the present study. Moreover, the total plasma testosterone concentration in infertile men was not significantly different from normal in spite of increased LH and SHBG binding capacity. This implies that there is a significant fall in testosterone secretion rate and unbound testosterone concentration, confirming the presence of Leydig cell dysfunction in infertile men despite normal total plasma testosterone levels.

That both FSH, and to a lesser extent LH, remain elevated in the infertile patients also implies that the rise in oestrogen is modest in magnitude or alternatively, that oestradiol plays a minor role in the feedback control of gonadotrophins. While compelling evidence has been accumulated in recent years supporting the existence of inhibin (Baker *et al.*, 1976; Franchimont *et al.*, 1979) its physiological role in humans in relation to gonadal steroids remains unresolved. Several experimental studies had demonstrated that testosterone, with or without oestradiol, provides the major negative feedback signal for FSH as well as LH secretion (Resko *et al.*, 1977; Plant *et al.*, 1978; Morris & Jackson, 1978; Main *et al.*, 1980). There is evidence also that oestrogens alone may preferentially suppress FSH (Kulin & Reiter, 1972; Sherins & Loriaux, 1973; Santen 1977; Sawin *et al.*, 1978; Lacroix *et al.*, 1979) and hence act synergistically with inhibin and/or testosterone (Resko *et al.*, 1977). Since inhibin-like activity resides in the aqueous extracts of testes (McCullagh, 1932), it is interesting to consider the possible role of oestrone sulphate, a water-soluble oestrogen conjugate, in the negative feedback control of FSH. Our results however demonstrated a positive rather than negative correlation between oestrone sulphate (and the unconjugated oestrogens) and FSH. It seems unlikely therefore that oestrogens have a major physiological role in the feedback control of FSH secretion in man.

ACKNOWLEDGEMENT

This study was conducted while FCWW was an MRC Clinical Training Fellow at the University of Edinburgh.

REFERENCES

Aloysio, D., Busacci, P., Bolelli, G.F., Vechi, F. & Flamigni, C. (1974) Radioimmunological assay of testosterone and gonadotrophins in plasma and seminal fluid of healthy subjects and patients with dysspermia. *Acta Europaea Fertilitatis*, **5**, 317–351.

Anderson, D.C., Lasley, B.L., Fisher, B.A., Shepherd, J.H., Newman, L. & Hendrickx, A.G. (1976) Transplacental gradients of sex-hormone-binding-globulin in human and simian pregnancy. *Clinical Endocrinology*, **5**, 657–669.

Baird, D.T., Horton, R., Longcope, C. & Tait, J.F. (1969) Steroid dynamics under steady-state conditions. *Recent Progress in Hormone Research*, **25**, 611–664.

Baird, D.T., Galbraith, A., Fraser, I. & Newsam, J.E. (1973) The concentration of oestrone and oestradiol-17β in spermatic venous blood in men. *Journal of Endocrinology*, **57**, 285–288.

Baker, H.W.G., Burger, H.G., de Kretser, D.M., Hudson, B. & Straffon, W.G. (1973) Effects of oral oestrogens in normal men and patients with prostatic carcinoma. Lack of gonadotrophin suppression by chlorotrianesine. *Clinical Endocrinology*, **2**, 297–306.

MIGEON, C.J., RIVAROLA, M.A. & FOREST, M.G. (1968) Studies of androgens in transsexual subjects. Effects of oestrogen therapy. *Bulletin of the Johns Hopkins Hospital*, **123,** 128–133.

MORRIS, I.D. & JACKSON, C.M. (1978) Gonadotrophic response after castration and selective destruction of the testicular interstitium in the normal and aspermatogenic rat. *Acta Endocrinologica*, **88,** 38–47.

MCCALLAGH, D.R. (1932) Dual endocrine activity of the testis. *Science*, **76,** 19–20.

NANKIN, H.R., CASTENEDA, E. & TROEN, P. (1977) Endocrine profiles in oligospermic men. In *The Testis in Normal and Infertile Men* (eds P. Troen & H.R. Nankin), pp. 529–537. Raven Press, New York.

NIESCHLAG, E., WICKINGS, E.J. & MAUSS, J. (1978) Endocrine testicular function in infertility. In *Recent Progress in Andrology*. Proceedings of Serono Symposium: Vol. 14, (eds A. Fabbrini & E. Steinberger), pp. 101–113. Academic Press, London.

NIESCHLAG, E., WICKLINGS, E.J. & MAUSS, J. (1979) Endocrine testicular function *in vivo* and *in vitro* in infertile men. *Acta Endocrinologica*, **90,** 544–551.

OSHIMA, H., NANKIN, H.R., TROEN, P., YOSHIDA, K.T. & OCHINAI, K.I. (1977) Leydig cell number and function in infertile men. In *The Testis in Normal and Infertile Men* (eds P. Troen & N.H. Nankin), pp. 445–465. Raven Press, New York.

PAYNE, A.H., KELCH, R.P., MUSICH, S.S. & HALPERN, M.E. (1976) Intratesticular site of aromatization in the human. *Journal of Clinical Endocrinology and Metabolism*, **42,** 1081–1087.

PEARLMAN, W.H., CREPY, O. & MURPHY, M. (1967) Testosterone-binding levels in the serum of women during the normal menstrual cycle, pregnancy and the post-partum period. *Journal of Clinical Endocrinology and Metabolism*, **27,** 1012–1018.

PLANT, T.M., HESS, D.L., HOTCHKISS, J. & KNOBIL, E. (1978) Testosterone and the control of gonadotropin secretion in the male rhesus monkey (*Macaca mulatta*). *Endocrinology*, **103,** 535–541.

PURVIS, K., BRENNER, P.E., LANDGREN, B-M., CEKAN, Z. & DICZSFALUSY, E. (1975) Indices of gonadal function in the human male. I. Plasma levels of unconjugated steroids and gonadotrophins under normal and pathological conditions. *Clinical Endocrinology*, **4,** 237–246.

RESKO, A.J., QUADRAI, S.K. & SPIES, H.G. (1977) Negative feedback control of gonadotropins in male rhesus monkeys: effects of time after castration and interactions of testosterone and oestradiol-17β. *Endocrinology*, **101,** 215–224.

RICH, K.A., KERR, J.B. & DE KRETSER, D.M. (1979) Evidence of Leydig cell dysfunction in rats with seminiferous tubular damage. *Molecular and Cellular Endocrinology*, **13,** 123–135.

RODRIGUEZ-RIGAU, L.J., WEISS, D.B., SMITH, K.D. & STEINBERGER, E. (1978) Suggestion of abnormal testicular steroidogenesis in some oligospermic men. *Acta Endocrinologica*, **87,** 400–412.

ROMMERTS, F.F.G., KRÜGER-SEWNARIAN, B.C., VAN-WOERKOM-BLIK, A., GROOTGOED, J.A. & VAN DER MOLEN H.J. (1978) Secretion of proteins by Sertoli cell enriched cultures: effects of follicle stimulation hormone, dibutryl cAMP and testosterone and correlation with secretion of oestradiol and androgen binding protein. *Molecular and Cellular Endocrinology*, **10,** 39–53.

ROMMERTS, F.F.G., KRÜGER-SEWNARIAN, B.C., GROOTGOED, J.A. DE JONG, F.H. & VAN DER MOLEN, H.J. (1979) Secretion of protein, androgen binding protein and oestradiol by Sertoli cells in culture: effects of serum. *Acta Endocrinologica*, **90,** 552–561.

ROSEN, S.W. & WEINTRAUB, B.D. (1971) Monotropic increase of serum FSH correlated with low sperm count in young men with idiopathic oligospermia and aspermia. *Journal of Clinical Endocrinology and Metabolism*, **32,** 410–416.

RUDER, H.J., LORIAUX, D.L., SHERINS, R.J. & LIPSETT, M.B. (1974) Leydig cell function in men with disorder of spermatogenesis. *Journal of Clinical Endocrinology and Metabolism*, **38,** 244–247.

RUDER, H.J., LORIAUX, D.L. & LIPSETT, M.B. (1972) Estrone sulphate: Production rate and metabolism in man. *Journal of Clinical Investigation*, **51,** 1020–1033.

SANTEN, R.J. (1977) Independent effects of testosterone and oestradiol on the secretion of gonadotrophin in man. In *The Testis in Normal and Infertile Men* (eds H.R. Nankin & P. Troen), pp. 197–211. Raven Press, New York.

SAWIN, C.T., RYAN, R.J., LONGCOPE, C. & FISHER, L.E. (1978) Effect of chronic administration of oestrogen, androgen or both on serum levels of gonadotrophins in adult men. *Journal of Clinical Endocrinology and Metabolism*, **46,** 911–915.

SHERINS, R.J. & LORIAUX, D.L. (1973) Studies on the role of sex steroids in the feedback control of FSH concentration in men. *Journal of Clinical Endocrinology and Metabolism*, **36,** 886–893.

SITTERII, P.K. & MACDONALD, P.C. (1973) Role of extraglandular estrogen in human endocrinology. In *Handbook of Physiology. Section 7. Vol. II*, (eds. R.I. Greep & E.B. Astwood), pp. 615–630. American Physiological Society, Washington.

under general anaesthesia, blood was aspirated by needle puncture simultaneously from the left spermatic vein (2–10 ml) and the antecubital vein (10–20 ml). The plasma was separated by centrifugation and stored at −20 °C until analysis. Blood samples were taken between 09.00 and 14.00 h whilst patients were in a horizontal position.

Radioimmunoassay for oestrone sulphate and oestrone

Radioactive oestrogens

[6,7-^{3}H]Oestrone-3-sulphate potassium salt (sp. act. 39 Ci/mmol) and [2,4,6,7-^{3}H]oestrone (sp. act. 110 Ci/mmol) from The Radiochemical Centre, Amersham were used without further purification.

Non-radioactive oestrogens

Oestrone-3-sulphate potassium salt containing 9·6% by weight of potassium acetate stabilizer was obtained from Sigma (London) Ltd, Poole. The purity was confirmed by thin-layer chromatography before use. Solutions were made up in phosphate–gelatin buffer and used within 1 week. Solutions of oestrone (Calbiochem C.P. Laboratories Ltd, Bishop Stortford) were made up in absolute ethanol and stored at 4 °C.

Solvents and reagents

Organic solvents were AR grade (BDH Ltd, Poole). Diethyl ether was washed with acidified ferrous sulphate solution (180 mmol ferrous sulphate in 100 ml 5% (w/v) sulphuric acid) and redistilled before use. Sterile distilled water was purchased from Antigen Ltd, Rosecrea, Ireland. Steroid-free plasma was prepared by treatment with charcoal (Norit A; Sigma) of plasma obtained from female blood-donors.

Phosphate–gelatin buffer (pH 7) was prepared by dissolving NaN_3 (77 mmol), NaCl (770 mmol), Na_2HPO_4 (305 mmol), $NaH_2PO_4.H_2O$ (173 mmol) and gelatin (5 g) in deionized distilled water (5 litres). Dextran-coated charcoal suspension contained 250 mg charcoal (Norit A; Sigma) and 25 mg dextran T70 (Pharmacia G.B. Ltd, Hounslow) in 100 ml phosphate–gelatin buffer.

Internal standards for estimation of procedural loss

[^{3}H]Oestrone sulphate was added to each plasma sample for assessment of procedural loss. For the oestrone estimations, three to four samples containing 1 ml male plasma and [^{3}H]oestrone (5000 counts/min, 37 fmol) were processed in each assay for estimation of group recovery. The mean recovery in oestrone sulphate samples ($n = 173$) was $63{\cdot}4 \pm 8{\cdot}8\%$ and in oestrone samples (eight assays, $n = 30$) was $84{\cdot}3 \pm 5{\cdot}6\%$. After the addition of these internal standards, the samples were left at room temperature for 1 h to equilibrate.

Extraction of unconjugated oestrone before acid solvolysis

One millilitre of plasma was shaken twice with 5 ml freshly distilled peroxide-free diethyl ether. The organic phases were combined and dried under N_2 at 40 °C. The residue was dissolved in 100 µl phosphate–gelatin buffer and kept at −20 °C until assay.

Acid solvolysis

This procedure was based on the method of Hawkins & Oakey (1974) with minor modifications. After extraction of unconjugated oestrone, oestrone sulphate in each plasma sample was solvolysed by adding 7·6 mmol crystalline $(NH_4)_2SO_4$, 25 µl 66% aqueous (v/v) sulphuric acid and 5 ml ethyl acetate. The reaction mixture was left overnight or for a minimum of 16 h in a water bath at 43 °C. The unconjugated oestrone released by solvolysis was extracted into diethyl ether as above. The ether was washed with 1 ml 8% (w/v)

The gonadotrophins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), were measured by the method of Hunter & Bennie (1979). The LH standard 68/40 (77 units/ampoule) and FSH standard 69/104 (10 units/ampoule) from the National Institute of Biological Standards and Control, London were used. Within-assay variations for LH and FSH were 8·0 and 7·0% respectively. Interassay variations were 8·6 and 7·4% for LH and FSH respectively. The limit of detection was 0·65 units/l for LH and 0·74 units/l for FSH.

Semen analysis

Semen analysis was carried out in the eight patients who presented with infertility. The method of analysis was as described in Chandley, Edmond, Christie, Gowans, Fletcher, Frackiewicz & Newton (1975). At least two semen samples from each of these patients were analysed and the average sperm density was used.

Statistical analyses

Statistical analyses were performed using Student's one-tailed *t*-test and Kendall's rank correlation.

RESULTS

Validation of assays for oestrone sulphate and oestrone

Specificity

The specificity of the assay of oestrone sulphate was dependent on the solvolysis step and the specificity of the antiserum. Cross-reactions with other steroids, expressed relative to oestrone (100%) at 50% tracer binding in the displacement of the dose–response curve (Abraham, 1969), were as follows: oestrone sulphate, 30·5%; equilenin, 15·0%; oestradiol-17β, oestradiol-17α, oestriol, dehydroepiandrosterone, testosterone, androsterone, 17α-hydroxyprogesterone, progesterone, cortisol and aldosterone, all less than 0·02%.

There was no significant difference in the mean concentrations of oestrone sulphate measured with and without celite column chromatography in replicate estimates of plasma from postmenopausal women ($0{\cdot}71 \pm 0{\cdot}10$ *v.* $0{\cdot}63 \pm 0{\cdot}10$ nmol/l, $n = 8$, $P > 0{\cdot}1$) and normal men ($1{\cdot}96 \pm 6{\cdot}29$ *v.* $1{\cdot}82 \pm 0{\cdot}04$ nmol/l, $n = 8$, $P > 0{\cdot}1$).

Accuracy

Known amounts of oestrone sulphate (potassium salt) and oestrone were added to water and samples of human plasma. The slopes of the regression equations were not significantly different from unity indicating that there were no systematic errors in the method. For oestrone sulphate added to water: amount found = 0·99(amount added) + 0·13 nmol/l; for oestrone sulphate added to plasma from postmenopausal women: amount found = 0·97(amount added) + 1·25 nmol/l; for oestrone added to plasma from pre-menopausal women: amount found = 0·96(amount added) + 0·27 nmol/1. The *y* intercept for oestrone sulphate added to water was not significantly higher than that for the average water blank in subsequent assays (0·05 nmol/1).

Precision

The within-assay precision for the oestrone sulphate assay estimated from assay of 25 duplicate samples (range of concentration 0·08–4·30 nmol/1) in four assays was 6·7%. For the oestrone assay, the within-assay precision was determined in replicate samples at four different concentrations, 126 ± 9, 252 ± 17, 474 ± 44 and 978 ± 57 pmol/l, giving coefficients of variation of 6·9, 6·9, 9·3 and 5·8% respectively, with a mean of 7·2%.

Interassay precision was assessed by repeated analyses of three plasma samples with mean concentrations ($\pm$S.D.) of $1{\cdot}27 \pm 0{\cdot}10$, $2{\cdot}46 \pm 0{\cdot}28$ and $5{\cdot}68 \pm 1{\cdot}36$ nmol/l for oestrone

sulphate and 123 ± 6, 160 ± 12 and 264 ± 66 pmol/l for oestrone in three to five consecutive assays. The average interassay coefficients of variation for the oestrone sulphate and the oestrone assays were 15 and 12·5% respectively.

Sensitivity

The average binding in the zero standard tubes was 60·0 ± 3·9% of total oestrone tracer added (5000 counts/min = 38 fmol/tube) in eight consecutive assays. The sensitivity of the standard curve (12·6 fmol), defined as the smallest amount of steroid significantly different from zero, was derived from the standard deviation of zero points of standard curves. Taking into account the procedural losses (mean recoveries for oestrone sulphate 63·4% and oestrone 84·3%) and the volume of plasma extract used for assay, the sensitivities of the assays were 0·1 nmol/l for oestrone sulphate and 15 pmol/l for oestrone.

In the oestrone sulphate assay, the mean (±S.D.) value for the water blank was 0·05 ± 0·01 nmol/l ($n = 4$) and for the average plasma blank, 0·11 ± 0·01 nmol ($n = 3$). The range of values obtained for experimental samples was 0·48–5·42 nmol/l. The lower limit of detection of the method was arbitrarily set at 0·14 nmol/l, equivalent to 80% inhibition of the binding found at zero mass. The plasma and water blank values were consistently less than the detection limit and were not subtracted from the results.

In the oestrone assay, the mean (±S.D.) blank value for water was 30·7 ± 7·4 pmol/l ($n = 5$) and for steroid-free plasma was 50·7 ± 4·4 pmol/l ($n = 3$). The working range in this study for oestrone was 111–760 pmol/l. The lower limit of detection of the method was set at 55 pmol/l, equivalent to 70% inhibition of the binding at zero mass. Blank values were not subtracted from the results.

Concentration of steroids in spermatic venous plasma

The concentrations of the five steroids in the spermatic and peripheral venous plasma from individual subjects are listed in Table 1. The concentrations of testosterone, androstenedione, oestradiol and oestrone were significantly higher in plasma from the spermatic vein than from the peripheral vein ($P < 0·001$, $P < 0·005$, $P < 0·025$, $P < 0·01$ respectively, paired t-test). The ratios of the mean concentrations in spermatic venous plasma to those in peripheral venous plasma were 77·2, 9·1, 28·7 and 1·6 respectively. Although the mean concentration of oestrone sulphate in the spermatic vein was significantly higher ($P < 0·05$, paired t-test) than that in peripheral vein, the ratio of the mean spermatic and peripheral concentrations was not significantly different from unity. Furthermore, in five patients the spermatic vein concentrations were lower than those in the peripheral vein.

There were significant correlations ($P < 0·05$ to $< 0·01$, Kendall's rank correlation) between the testicular secretion, as represented by the individual spermatic : peripheral venous concentrations in each of the 17 patients, of oestrone, oestradiol, androstenedione and testosterone but not of oestrone sulphate.

Although the mean LH and FSH levels in the 17 patients with varicocoeles were within the appropriate normal ranges (LH 3·4–10·0 units/l and FSH 1·7–6·7 units/l), four patients (nos 1, 4, 6 and 10), one of whom was severely oligospermic, had raised concentrations of FSH. The mean levels of various steroids in spermatic and peripheral venous blood in these four patients with raised FSH were not significantly different from those with normal gonadotrophins although the mean spermatic vein concentration of oestradiol was higher in the former group (8·9 ± 6·0 *v.* 3·7 ± 6·0 nmol/l).

DISCUSSION

The estimation of oestrone sulphate was carried out with an antiserum directed against oestrone and applied to the assay of oestrone released after acid solvolysis of plasma samples. This approach was selected because of the difficulty in raising an effective antiserum against oestrone sulphate due to the instability of the immunogen. Specificity of

testicular hormone secretion is low, as for oestrone, these variables may have disproportionate effects leading to underestimation. There does not seem to be any entirely satisfactory explanation for the discrepant results in testicular secretion of oestrone at present.

Although some studies claimed to have demonstrated abnormal Leydig cell function in patients with varicocoeles, this has not been confirmed by others. Raboch & Starka (1971) and Comhaire & Vermeulen (1975) found lower peripheral testosterone levels in patients with varicocoeles which correlated with increasing age. In-vitro studies using testicular biopsy specimens showed reduced synthesis of [^{3}H]testosterone from [^{3}H]pregnenolone in 16 patients with varicocoeles compared with one normal control (Weiss, Rodriguez-Rigau, Smith & Steinberger, 1978). However, normal concentrations of gonadotrophins, testosterone and oestradiol were reported by Swerdloff & Walsh (1975), Schiff, Wilson, Newton, Shane, Kates, Ryan & Naftolin (1976) and Rege, Phadke, Bhatt, Khatri, Sheth, Joski & Vaidya (1979). Baird *et al.* (1973) and Swerdloff & Walsh (1975) also found comparable levels of testosterone and oestradiol in spermatic veins of patients with varicocoeles or inguinal hernias. In the present study, comparison of spermatic and peripheral vein concentrations of all five steroids between the four patients with raised FSH and/or oligospermia and the rest of the study group with normal FSH and/or sperm densities showed only higher spermatic vein concentrations of oestradiol which did not reach the 5% level of statistical significance. The balance of available evidence would suggest that studies on testicular steroid secretion based on spermatic vein concentrations in patients with varicocoeles may justifiably be extrapolated to the normal physiological situation. However, other factors which have not so far been taken into account are the suppressive effects of surgical stress (Eik-Nes, 1970; Wang, Chan & Yeung, 1978) and anaesthesia (Carstensen, Amen, Wide & Amen, 1973) on Leydig cell function.

This study was carried out while F. C. W. W. was an MRC Clinical Training Fellow at the Department of Obstetrics and Gynaecology, University of Edinburgh. We wish to thank Mr J. W. Fowler, Department of Urology, Royal Infirmary, Edinburgh, for his co-operation in obtaining some of the spermatic venous samples and Dr R. A. Hawkins, Department of Clinical Surgery, University of Edinburgh for his encouragement and advice.

REFERENCES

Abraham, G. E. (1969). Solid-phase radioimmunoassay of estradiol-17β. *Journal of Clinical Endocrinology and Metabolism* **29**, 866–870.

Baird, D. T., Burger, P. E., Heavon-Jones, G. D. & Scaramuzzi, R. J. (1974). The site of secretion of androstenedione in non-pregnant women. *Journal of Endocrinology* **63**, 201–212.

Baird, D. T., Galbraith, A., Fraser, I. & Newsam, J. E. (1973). The concentration of oestrone and oestradiol-17β in spermatic venous blood in man. *Journal of Endocrinology* **57**, 285–288.

Baird, D. T., Horton, R., Longcope, C. & Tait, J. F. (1968). Steroid prehormones. *Perspectives in Biology and Medicine* **11**, 384–421.

Baird, D. T., Horton, R., Longcope, C. & Tait, J. F. (1969). Steroid dynamics under steady-state conditions. *Recent Progress in Hormone Research* **25**, 611–656.

Baulieu, E. E., Corpechot, C., Dray, F., Emiliozzi, R., Lebeau, M.-C., Mauvais-Jarvis, P. & Robel, P. (1965). An adrenal-secreted 'androgen': dehydroepiandrosterone sulphate. Its metabolism and a tentative generalization on the metabolism of other steroid conjugates. *Recent Progress in Hormone Research* **21**, 411–500.

Brown, J. B. & Smyth, B. J. (1971). Oestrone sulphate–the major circulating oestrogen in the normal menstrual cycle? *Journal of Reproduction and Fertility* **24**, 142.

Carstensen, H., Amen, I., Wide, L. & Amen, B. (1973). Plasma testosterone LH and FSH during the first 24 h after surgical operations. *Journal of Steroid Biochemistry* **4**, 605–611.

Chandley, A. C., Edmond, P., Christie, S., Gowans, L., Fletcher, J., Frackiewicz, A. & Newton, M. (1975). Cytogenetics and infertility in men. I. Karotype and seminal analysis. *Annals of Human Genetics* **39**, 231–254.

de la Torre, B., Noren, S., Hedman, M. & Diczfalusy, E. (1978). Studies on the relationship between sperm count and steroid levels in the spermatic and cubital veins of patients with varicocoeles. *International Journal of Andrology* **1**, 297–307.

Van Look, P. F. A., Hunter, W. M., Corker, C. S. & Baird, D. T. (1977). Failure of positive feedback in intact men and in male pseudohermaphrodites with the syndrome of testicular feminization. *Clinical Endocrinology* **7**, 353–366.

Wang, C., Chan, V. & Yeung, R. T. T. (1978). Effects of surgical stress on pituitary–testicular function. *Clinical Endocrinology* **9**, 255–266.

Weinstein, R. L., Kelch, R. B., Jenner, M. R., Kaplan, S. L. & Grumbach, M. M. (1974). Secretion of unconjugated androgens and estrogens by the normal and abnormal human testis before and after human chorionic gonadotropin. *Journal of Clinical Investigation* **53**, 1–6.

Weiss, D. B., Rodriguez-Rigau, L. J., Smith, K. D. & Steinberger, E. (1978). Leydig cell function in oligospermic men with varicocoele. *Journal of Urology* **120**, 427–430.

Wright, K., Collins, D. C., Musey, P. I. & Preedy, J. R. K. (1978). A specific radioimmunoassay for estrone sulfate in plasma and urine without hydrolysis. *Journal of Clinical Endocrinology and Metabolism* **47**, 1092–1098.